

# **APPENDIX A**

## SECTION 14

# Regular, Nonsporing Gram-Positive Rods

Otto Kandler and Norbert Weiss

**Table 14.1**  
**Differential properties of the regular nonsporing rods\***

Characteristics	Catalase-negative facultative anaerobes		Catalase-positive facultative anaerobes		Aerobes		
	<i>Lactobacillus</i>	<i>Erysipelothrix</i>	<i>Brochothrix</i>	<i>Listeria</i>	<i>Kurthia</i>	<i>Coryophanon</i>	<i>Renibacterium</i>
Cell morphology	Rods, usually straight, sometimes coccobacilli	Slender rods, often filaments	Slender rods, often filaments	Short rods, often short chains & filaments	Regular rods in chains, cocci in old cultures	Short rods in chains	Short rods, often in pairs
Multicellular rods (trichomes)	—	—	—	—	—	+	—
Diameter of rods, microns	0.5-1.1*	0.2-0.5	0.6-0.8	0.4-0.5	0.7-0.9	1.4-3.2	0.3-1.0
Motile (if motile, peritrichate flagella)	—	—	—	+	+	+	—
Strictly aerobic	—	—	—	—	+	+	+
Facultatively anaerobic or microaerophilic	+	+	+	+	—	—	—
Catalase reaction	—	—	+	+	+	+	+
Major fermentation products from carbohydrates anaerobically (if NA, acidity from glucose is noted)	Mainly lactate, but may give some acetate, ethanol, CO <sub>2</sub>	Lactate	Mainly lactate	Lactate	NA (no acid)	NA (no acid)	NA (no acid)
Peptidoglycan group†	A	B	A	A	A	A	A
Peptidoglycan: type of diamino acid‡	Lys, mDAP, Orn	Lys	mDAP	mDAP	Lys	Lys	Lys
Major fatty acids§	S, U, sometimes C	S, A, I, U	S, A, I	S, A, I	S, A, I	ND	A
Major menaquinone¶	None	None	MK-7	MK-7	MK-7	MK-6	MK-9
Habitat	Widespread in fermentable materials, very rarely pathogenic	Widespread pathogen in vertebrates	Meat products, nonpathogenic	Widespread in decaying material, may be vertebrate pathogen	Feces of farm animals, meat products, non-pathogenic	Cow dung, non-pathogenic	Pathogen in salmonid fish
Mol% G + C of DNA	32-53	36-40	36	36-38	36-38	41-46	53

\* Symbols: +, 90% or more of strains are positive; —, 90% or more of strains are negative; NA, not applicable; and ND, not determined.

† Sometimes up to 1.6 µm.

‡ Rarely motile.

§ At 20-25°C; poorly motile at 37°C.

¶ Numerous flagella.

‡ Rhizoid colonies.

\* Some strains weak positive.

\* Symbolism of Schleifer & Kandler (1972).

† Kuster and Fiedler (1983).

‡ S, straight-chain saturated; U, monounsaturated; A, anteiso-methyl-branched; I, iso-methyl-branched; C, cyclopropane ring fatty acids.

¶ Collins (1982).

‡ Collins and Jones (1981).

\* *L. mali* contains MK-8 and MK-9; a menaquinone is also found in *L. casei* subsp. *rhannonius*.

This section comprises a conglomerate of seven very different genera (Table 14.1) which have in common only a few morphological and physiological characteristics. They are all rod-shaped cells, (coccoid to elongated rods, filaments or trichomes) Gram-positive, nonsporing, nonpigmented (slight yellow pigmentation in *Coryophanon*), mesophilic, chemoorganotrophic, and grow only in complex media.

The largest genus, *Lactobacillus*, which is well characterized, with either homo- or heterolactic fermentation, comprises about 50 species, whereas each of the other six genera is monospecific or contains only a few (up to five) species. Most of the genera in the group exhibit unique characteristics which facilitate their differentiation and identification. The genus *Coryophanon* is easily recognized by the formation of trichomes consisting of disk-like cells, 1.5-2.0 µm wide and only 0.5-

1.0 µm long. *Coryophanon* is also well characterized by its habitat, cow feces, where it grows abundantly one to several days after the feces is voided.

The two species of the genus *Kurthia*, also commonly found in feces of farm animals, are recognizable by the characteristic "Medusa-head" appearance of their colonies on yeast extract nutrient agar, and "birds-feather" growth in nutrient gelatin.

Two monospecific genera cause unique diseases. *Erysipelothrix* is well known as the causative organism of swine erysipelas, and *Renibacterium* is an obligate pathogen of the subfamily Salmoninae of the salmon family, causing nephrotic syndromes. Species of the genus *Listeria* (e.g. *L. monocytogenes*) are characteristic pathogens involved in several inflammatory infections (listeriosis) in humans and animals.

Saprophytic species of *Listeria* are wide-spread in soil and decaying matter. They are often isolated from meat and meat products and may thus be confused with species of *Brochothrix* and *Kurthia*, nonpathogenic saprophytes also common in this habitat.

The differentiation of the facultatively anaerobic *Listeria*, containing meso-diaminopimelic acid in its peptidoglycan, from *Kurthia* is relatively easy, because *Kurthia* is strictly aerobic and possesses a lysine-containing peptidoglycan (Table 14.1). *Brochothrix*, however, shares numerous morphological and biochemical characteristics with *Listeria*. Therefore, the differentiation of these two genera is mainly based on differences in motility (Table 14.1) and minor physiological characteristics, e.g. inability of *Brochothrix* to grow at 37°C, pattern of fermented sugars, etc.

Metabolically, the seven genera may be divided into three groups: Group 1 consists of the two fermentative, saccharolytic, microaerophilic genera *Lactobacillus* and *Erysipelothrix*. They do not possess heme-containing catalase, cytochromes or menaquinones and they utilize oxygen only via flavin-containing oxidases and peroxidases.

Group 2 comprises the two aerobic, and facultatively anaerobic genera *Brochothrix* and *Listeria* which possess cofactors and enzymes for respiration. However, these organisms are also able to ferment sugars, mainly to lactic acid, under oxygen-limited or anaerobic conditions.

Group 3 contains the three strictly aerobic genera *Kurthia*, *Corynebacterium* and *Renibacterium* which neither utilize glucose as carbon or energy source nor ferment sugars to organic acids.

These groupings have only limited taxonomic value as indicated by the low correlation with nonmetabolic characteristics. In fact, four genera, *Brochothrix* (formerly *Microbacterium thermosphactum*), *Listeria*, *Kurthia* and *Erysipelothrix*, have often been associated with the *Corynebacteriaceae* or at least with the coryneform group (Bergey 7). However, numerical taxonomic and chemotaxonomic studies have not supported this affiliation. Such studies rather suggest a remote relationship between coryneform organisms and the lactic acid bacteria (Wilkinson and Jones 1977). The presence of respiratory cofactors and enzymes in *Listeria*, *Brochothrix* and *Kurthia* is not in keeping with their inclusion within an enlarged family *Lactobacillaceae* (Collins et al., 1979). However the genera *Brochothrix*, *Listeria*, *Lactobacillus* and *Erysipelothrix* are close phenetically to each other and to *Streptococcus* and *Gemella* (Wilkinson and Jones, 1977).

The G + C ratios of the DNA of six of the seven genera fall within a range around 40 mol% (*Lactobacillus fermentum* is 50 mol%), whereas with *Renibacterium* 53 mol% is found. Comparative studies on the sequence homology of 16S-rRNA oligonucleotides in a large number of bacteria of different taxonomic affiliation showed that all the Gram-positive bacteria possessing a G + C content lower than about 55 mol% belong to the so-called *Clostridium-Lactobacillus-Bacillus* branch (Fox et al., 1980, Stackebrandt and Woese, 1981). In fact, detailed studies

on the 16S-rRNA oligonucleotides of representatives of the six genera showed that they fit into this branch.

From the rRNA evidence the lactobacilli and streptococci together with the pediococci and leuconostocs are close to the genera *Bacillus*, *Brochothrix*, *Listeria*, *Staphylococcus*, *Gemella* and *Kurthia*. This position reflects the metabolism of the lactic acid bacteria, which is intermediate between aerobic and anaerobic metabolism. *Listeria* and *Brochothrix* are closely related to one another, and together with *Staphylococcus* are closer to *Bacillus* than are the lactic acid bacteria.

On the basis of 16S-rRNA cataloging, *Erysipelothrix* is related to the mycoplasmas, exhibiting nonisochronic evolution of their 16S-rRNA sequences (Ludwig et al., 1984). Thus, *Erysipelothrix* represents one of the many different lines of nonrespiratory organisms emerging from the *Clostridium* cluster. One of these lines comprises *Eubacterium limosum*, *Acetobacterium woodii* and *Clostridium barkeri* (Fox et al., 1980) which, in addition to other common properties, are characterized by the same very unusual peptidoglycan types of the cross-linking group B (Schleifer and Kandler, 1972), also found in *Erysipelothrix*. Position one of the peptide subunits of these group B peptidoglycan types is taken by a L-seryl residue, not by L-alanyl as in group A peptidoglycans, or by a glycyl residue as in those group B peptidoglycans occurring only in a certain section of the coryneform bacteria (*Arthrobacter*, *Clavibacter*, *Curtobacterium*, *Microbacterium*). Thus, comparative peptidoglycan chemistry corroborates the affiliation of *Erysipelothrix* with the *Clostridium* cluster which also harbors the *Eubacterium limosum* line, while the above mentioned group B peptidoglycan-containing coryneform genera belong to the *Actinomycetes* branch.

No data on 16S-rRNA cataloging are available for *Corynebacterium* and *Renibacterium*. The low G + C content of the DNA of *Corynebacterium* suggests an affiliation also with the *Clostridium-Lactobacillus-Bacillus* branch. However, the fairly high G + C content of 53 mol% in *Renibacterium* falls within the overlapping zone of the *Clostridium* and the *Actinomycetes* branch. Thus *Renibacterium* could be allotted to either branch. Phenetically, *Renibacterium* resembles the genus *Arthrobacter* in morphology, "Chinese letter" formation, slightly yellow pigmentation of colonies, aerobic metabolism, the presence of MK-9 instead of MK-7 menaquinone, and in its unusual peptidoglycan type, containing D-alaninamide (Kuiser and Fiedler, 1983) found so far in only one other organism, *Arthrobacter* sp. NCIB 9423 (Fiedler et al., 1973). Therefore, *Renibacterium* is tentatively included in the *Actinomycetes* branch in Fig. 14.1 (see *Lactobacillus*). Final affiliation will only be possible on the basis of 16S-rRNA analysis or other sequence and chemotaxonomic or numerical taxonomic data.

In conclusion, the seven genera discussed in this section certainly do not belong to the same family. However, with the exception of *Renibacterium*, they may at present be allotted to the same order or superorder, in the event that the whole *Clostridium-Lactobacillus-Bacillus* branch may finally be recognized as a taxon at such a rank.

### Genus *Lactobacillus* Beijerinck 1901, 212<sup>AL</sup>

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*Lac.to.ba.cil'lus*. L. n. *lac*, *lactis* milk; L. dim. n. *bacillus* a small rod; M.L. n. *Lactobacillus* milk rodlet.

Cells, varying from long and slender, sometimes bent rods to short, often coryneform coccobacilli; chain formation common. Motility uncommon; when present, by peritrichous flagella. Nonsporing. Gram-positive. Some strains exhibit bipolar bodies, internal granulations or a barred appearance with the Gram-reaction or methylene blue stain.

Metabolism fermentative; obligately saccharoclastic. At least half of end product carbon is lactate. Lactate is usually not fermented. Additional products may be acetate, ethanol, CO<sub>2</sub>, formate or succinate. Volatile acids with more than two carbon atoms are not produced.

Microaerophilic; surface growth on solid media generally enhanced by anaerobiosis or reduced oxygen pressure and 5-10% CO<sub>2</sub>; some are anaerobes on isolation.

Nitrate reduction highly unusual; if present, only when terminal pH is poised above 6.0. Gelatin not liquefied. Casein not digested but small amounts of soluble nitrogen produced by most strains. Indole and H<sub>2</sub>S not produced.

Catalase and cytochrome negative (porphyrins absent); however, a few strains decompose peroxide by a pseudocatalase; benzidine reaction negative.

Pigment production rare; if present, yellow or orange-to-rust or brick red.

Complex nutritional requirements for amino acids, peptides, nucleic acid derivatives, vitamins, salts, fatty acids or fatty acid esters and fermentable carbohydrates. Nutritional requirements are generally characteristic for each species, often for particular strains only.

Growth temperature range 2–58°C; optimum generally 30–40°C.

Aciduric, optimal pH usually 5.5–6.2; growth generally occurs at 5.0 or less; the growth rate is often reduced at neutral or initially alkaline reactions.

Found in dairy products, grain products, meat and fish products, water, sewage, beer, wine, fruits and fruit juices, pickled vegetables, sauerkraut, silage, sour dough, and mash; they are a part of the normal flora in the mouth, intestinal tract and vagina of many homothermic animals including man. Pathogenicity is rare.

The mol% G + C of the DNA ranges from 32–53 (Bd, T<sub>1</sub>).

Type species: *Lactobacillus delbrueckii* (Leichmann 1898) Beijerinck 1901, 229.

#### Further Descriptive Information

**Cell morphology.** The variability of lactobacilli from long, straight or slightly crescent rods to coryneform coccobacilli is depicted in Figure 14.1. The length of the rods and the degree of curvature is dependent on the age of the culture, the composition of the medium—e.g. availability of oleic acid esters (Jacques et al., 1980)—and the oxygen tension. However, the main morphological differences between the species usually remain clearly recognizable. Some species of the gas-producing lactobacilli (e.g. *L. fermentum*, *L. brevis*) always exhibit a mixture of long and short rods (Fig. 14.1E).

Coccobacilli may become so short that they may be confused with

Figure 14.1. Phase contrast (A–E) and electron micrographs (F) showing different cell morphology of lactobacilli (A, *L. gasseri*; B, *L. agi*; C, *L. curvatus*; D, *L. minor*; E, *L. fermentum*; F, involution form of lactobacilli in a thin section of a kefir grain).

either *Leuconostoc* (e.g. *L. confusus*, originally considered as *Leuconostoc*) or streptococci. On the other hand, elongated streptococci have repeatedly been ascribed to the genus *Lactobacillus*, e.g. *L. xylosum* and "*L. horvathiae*," recently found, to belong to the genus *Streptococcus* (Garvie et al., 1981; Kipper-Bälz et al., 1982). Cell division occurs only in one plane. The tendency towards chain formation varies between species and even strains. It depends on the growth phase and the pH of the medium (Rhee and Pack, 1980). The asymmetrical development of cells during cell division in coryneform lactobacilli (Fig. 14.2) leads to wrinkled chains or even ring formation. Irregular involution forms may be observed under symbiotic growth, e.g. in kefir grains (Fig. 14.1F) or under the influence of high concentrations of glycine, D-amino acids or cell wall-active antibiotics (Hammes et al., 1978; Schleifer et al., 1976). Motility by peritrichous flagellation is observed in only a few species. It is highly dependent on the medium and the age of the culture and is sometimes observed only during isolation, but lost after several transfers on artificial media.

All lactobacilli stain clearly Gram-positive. Only dying cells may give

variable results. Internal granulation is often revealed by Gram or methylene blue stain especially in the homofermentative long rods. The large bipolar bodies probably contain polyphosphate and appear very electron-dense in electron microscopy.

**Cell wall and fine structure.** Electron micrographs of thin sections reveal a typical Gram-positive cell wall profile (Figs. 14.2 and 14.3). The cell wall contains peptidoglycan (murein) of various chemotypes of the cross-linkage group A. The Lys-D-Asp type is the most widespread peptidoglycan type (Schleifer and Kandler, 1972). The cell wall contains also polysaccharides attached to peptidoglycan by phosphodiester bonds (Knox and Hall, 1984). Membrane-bound teichoic acid is present in all species (Archibald and Baddiley, 1966), cell wall-bound teichoic acid only in some of the species (Knox and Wicken, 1973). Extracellular slime in large amounts is produced from sucrose by *L. confusus* and particular strains of some other heterofermentative species (Sharpe et al., 1972). Slime-forming strains of *L. delbrueckii* subsp. *bulgaricus* and *L. casei* are employed for the production of special sour milks.

with (m,

plasmic

In addition to nucleoids and ribosomes typical of all procaryotes, electron micrographs of thin sections frequently show large mesosomes (Fig. 14.3). They are formed by invaginations of the cytoplasmic membrane and are filled with tubuli, probably derived from secondary membrane invaginations (Schödtz et al., 1965; Sriranganathan et al., 1973).

**Colony and cultural characteristics.** Colonies on agar media are usually small (2–5 mm), with entire margins, convex, smooth, glistening, and opaque without pigment. In rare cases they are yellowish or reddish. Some species form rough colonies. Distinctly slimy colonies are only formed by *L. confusus*. Clearing zones caused by exoenzymes are usually not observed when grown on agar media containing dispersed protein or fat. However, most strains exhibit slight proteolytic activity due to cell wall-bound or cell wall-released proteases and peptidases (Law and Kolstad, 1983) and a weak lipolytic activity due to predominantly intracellular lipases. Distinct starch degradation leading to clearing zones on starch plates is only observed in a few species (e.g. *L. amylophilus*, *L. amylovorans*). Growth in liquid media generally occurs throughout the liquid, but the cells settle soon after growth ceases. The sediment is smooth and homogeneous, rarely granular or slimy. Pellicles are never formed.

Lactobacilli do not develop characteristic odors when grown in common media. However, they contribute to the flavor of fermented food by producing various volatile compounds, such as diacetyl and its derivatives, and even  $H_2S$  and amines in cheese (Sharpe and Franklin, 1962; Law and Kolstad, 1983).

**Nutrition and growth conditions.** Lactobacilli are extremely fastidious organisms, adapted to complex organic substrates. They require not only carbohydrates as energy and carbon source, but also nucleotides, amino acids and vitamins. While pantothenic acid and nicotinic acid are—with the exception of a few strains—required by all species, thiamine is only necessary for the growth of the heterofermentative lactobacilli. The requirement for folic acid, riboflavin, pyridoxal phosphate and p-aminobenzoic acid is scattered among the various species, riboflavin being the most frequently required compound. Biotin and  $B_{12}$  are required by only a few strains. Although the pattern of vitamin heterotrophy is considered to be characteristic of particular species (Rogosa et al., 1961), deviating strains are common (Abo-Elnaga and Kandler, 1965; Ledesma et al., 1977). Vitamin-dependent strains are commonly in use for bioassays of vitamins and are listed in the catalogues of most culture collections. The pattern of the amino acid requirement also differs among species and even strains. By sequential mutagenesis, Morishita et al. (1974) were able to obtain quintuple mutants of *L. casei* which had lost their requirement for 5 amino acids. However, the mutants grew significantly slower and reverted frequently to their amino acid-dependent state when transferred back to the complete medium. Corresponding results were also obtained with four other species (Morishita et al., 1981).

These studies show that many—if not all—of the nutritional requirements of lactobacilli are the result of numerous minor defects within the genome, and that much of the information coding for the various biosynthetic pathways is still present in the chromosome. Thus, the multiple nutritional requirements of present-day lactobacilli reflect the stepwise natural selection of deficient mutants out of a chemoautotrophic population with a complement of biosynthetic pathways.

The various requirements for essential nutrients are normally met when the media contain fermentable carbohydrate, peptone, meat and yeast extract. Supplementations with tomato juice, manganese, acetate and oleic acid esters, especially Tween 80, are stimulatory or even essential for most species. Therefore, these compounds are included in the widely used MRS medium (De Man et al., 1960). Lactobacilli adapted to very particular substrates may require special growth factors. For instance D-isovalonic acid is necessary for rice wine (sake) spoilage organisms (Tamura, 1956) and a small peptide isolated from freshly prepared yeast extract was found to be required for luxuriant growth of *L. sanfrancisco* (Berg et al., 1981), the sour dough organism.

To meet the requirement of a still unknown growth factor, some of the original substrate must be added.

Lactobacilli grow best in slightly acidic media with an initial pH of 6.4–4.5. Growth ceases when pH 4.0–3.6 is reached, depending on the species and strain. Although most strains are fairly aerotolerant, optimal growth is achieved under microaerophilic or anaerobic conditions. Increased  $CO_2$  concentration (~5%) may stimulate growth.

Most lactobacilli grow best at mesophilic temperatures with an upper limit of around 40°C. Some also grow below 15°C and some strains even below 5°C. The so-called "thermophilic" lactobacilli may have an upper limit of 55°C and do not grow below 15°C. Really thermophilic lactobacilli growing above 55°C are as yet unknown.

**Metabolism.** Metabolically, lactobacilli are at the threshold of anaerobic-to-aerobic life. They possess efficient carbohydrate fermentation pathways coupled to substrate level phosphorylation. A second substrate level phosphorylation site is the conversion of carbamyl phosphate to  $CO_2$  and  $NH_4$ , the final step of arginine "fermentation," observed in most of the heterofermentative lactobacilli (cf. Abdal, 1979). However, only some of the species forming  $NH_4$  from arginine are able to grow on arginine as the only energy source. In addition to substrate-level phosphorylation, energy may be gained by the proton motive force generated by lactate efflux (Könings and Otto, 1983). Lactobacilli contain no isoprenoid quinones—except *L. yamadaensis* and *L. casei* subsp. *rhamnosus* (Collins and Jones, 1981)—and no cytochrome systems to perform oxidative phosphorylation. However, they possess flavine-containing oxidases and peroxidases to carry out the oxidation of  $NADH_2$  and  $O_2$  as the final electron acceptor. They are also able to perform a manganese-catalyzed scavenging of superoxide (Götz et al., 1980; Archibald and Fridovich, 1981), although they do not possess superoxide dismutase and catalase.

The main fermentation pathways for hexoses are the Embden-Meyerhof pathway converting 1 mol of hexose to 2 mol of lactic acid (homolactic fermentation) and the 6-phosphogluconate pathway, resulting in 1 mol  $CO_2$ , 1 mol ethanol (or acetic acid) and 1 mol lactic acid (heterolactic fermentation). Under aerobic conditions, most strains are able to reoxidize  $NADH_2$  with oxygen serving as the final electron acceptor, thus acetyl-CoA is not, or at least not completely, reduced to ethanol. Consequently, additional ATP is formed by substrate-level phosphorylation and varying ratios of acetic acid and ethanol are found, depending on the oxygen supply.

Pyruvate, intermediately formed in both pathways, may partly undergo several alternative conversions, yielding either the well-known aroma compound diacetyl and its derivatives or acetic acid (ethanol) with hexose limitation, the latter pathway may become dominant and the homolactic fermentation may be changed to a heterofermentation with acetic acid, ethanol and formic acids as the main products (DeVries et al., 1970; Thomas et al., 1979). Even lactate may partially be oxidized and broken down to acetic acid and formate or  $CO_2$  by various little known mechanisms (cf. Kandler, 1983). The conversion of glycerol to 1,3-propanediol with glucose serving as electron donor is a peculiar metabolic activity observed in *L. brevis* isolated from wine (Schödtz and Radler, 1984).

At the enzyme level, homo- and heterofermentative lactobacilli differ with respect to the presence or absence of FDP aldolase or phosphoketolase. Whereas the heterofermentative lactobacilli possess phosphoketolase but no aldolase, the obligate homofermentative ones possess FDP aldolase but no phosphoketolase. They are thus unable to ferment any of the pentoses, which are broken down by the heterofermenters via phosphoketolase, yielding equimolar amounts of lactic acid and acetic acid. However, one group of homofermentative lactobacilli, traditionally called "Streptobacteria" (Orla Jensen, 1919), possess an inducible phosphoketolase with pentoses acting as inducers. They are thus able to ferment pentoses upon adaptation to lactic acid and acetic acid, while hexoses are homofermentatively metabolized. Therefore, these lactobacilli must be called facultative heterofermenters (group II; see below). In rare cases, a homolactic fermentation of pentoses may be performed by lactic acid bacteria, as observed in some streptococci by Fukui et al. (1957) and in a so far undescribed lactobacillus (Barre, 1978). Such fermentations may involve the transformation of pentoses to hexoses via transaldolase and transketolase reactions followed by



glycolysis (Kandler, 1983) with lactic acid being the only fermentation product.

Carbohydrates may also contribute to other reactions: sucrose is not only a substrate for fermentation, but also for the formation of dextrans (slime) with the help of dextran sucrases, found in only a few species or strains. Fructose serves not only as a substrate for fermentation, but also as an electron acceptor and becomes reduced to mannitol by most heterofermentative lactobacilli. Correspondingly, glycerol is formed from triosephosphate and excreted into the medium by some heterofermentative strains.

The majority of saccharides and oligosaccharides are taken up with the help of specific permeases and are phosphorylated inside the cell. Oligosaccharides are split by the respective glycosidases prior to the phosphorylation of the resulting monosaccharides. However, at least lactose and galactose are taken up by some lactobacilli via the phosphoenolpyruvate-dependent phosphotransferase system (Chassy and Thompson, 1983). The lactose phosphate formed is split to glucose and D-galactose-6-phosphate. The latter is then metabolized via the p-tartrate-6-phosphate pathway (cf. Kandler, 1983). Little is known of the distribution of the various saccharide uptake mechanisms in the species of the genus *Lactobacillus*, although the presence or absence of such mechanisms determines the pattern of fermented sugars, an important characteristic for identification. Active transport of amino acids and peptides is also known (cf. Law and Kolstad, 1983). However, more information is available on streptococci than on lactobacilli.

Several organic acids, such as citric, tartaric and malic acids, are degraded via oxalacetate and pyruvate to  $\text{CO}_2$  and lactic or acetic acid (cf. Radler, 1975; Whiting, 1975). Detailed studies on the catalytic and regulatory properties of the DNA-dependent malic enzymes of lactic acid bacteria were performed by London et al. (1971). Alternatively, malic acid is split to  $\text{CO}_2$  and L-(+)-lactic acid in many lactobacilli by a multifunctional so-called "malolactic enzyme" with all intermediates remaining tightly bound to the enzyme complex (Radler, 1975).

Several amino acids are decarboxylated by lactobacilli, e.g. glutamic acid and tyrosine, but the decarboxylation product is not further metabolized (Blood, 1975).

Chlorogenic acid is hydrolyzed and the resulting quinic acid is reduced to (-)-dehydroshikimic acid by heterofermentative lactobacilli. It is further reduced to dihydroxycyclohexane-1'-carboxylic acid by homofermenters. Shikimic acid may be reduced to catechol by *L. plantarum*, which also converts p-coumaric acid to p-ethylphenol. The electron source of these reactions is lactate which becomes oxidized to  $\text{CO}_2$  and acetic acid (cf. Whiting, 1975).

The lactic acid formed by the various fermentation pathways possesses either the L- or the D-configuration depending on the stereospecificity of the lactate dehydrogenase present in the cells. Racemate may be formed when both L- and D-lactate dehydrogenase are present in the same cell, or in rare cases, by the action of an inducible lactate racemase in combination with a constitutive L-lactate dehydrogenase (Stetter and Kandler, 1973). Lactate dehydrogenases of the various species often differ from each other considerably, e.g. with respect to their electrophoretic mobility and their kinetic properties. Most enzymes are nonallosteric but some species contain allosteric L-lactate dehydrogenases with FDP and  $\text{Mn}^{2+}$  acting as effectors (Hensel et al., 1977; cf. Garvie, 1980).

**Mutagenesis.** Spontaneous and induced mutants of lactobacilli are frequently selected to obtain strains exhibiting characters useful for biochemical studies or biotechnological application. The well-known mutagens N-methyl-N'-nitro-N-nitrosoguanidine, ethylmethane sulfonate and ultraviolet (UV) light have been applied successfully (Morishita et al., 1981).

**Plasmids.** No lactobacillus strain is known to be transformable or transducible and genetic engineering via recombinant DNA cannot be done at present in lactobacilli. However, plasmids are frequently found (Smiley and Fryder, 1978; Vescovo et al., 1981). They are often linked with drug resistance (Ishiwa and Iwata, 1980; Vescovo et al., 1982) or lactose metabolism (Chassy et al., 1976). The conjugal self-transmission of a plasmid that determines lactose metabolism in *L. casei* is the only

known naturally occurring genetic exchange in the genus (Chassy and Rokow, 1981). Extensive research, including cloning in *Escherichia coli*, is proceeding with the plasmids coding lactose metabolism (Chassy et al., 1983) in order to make the lactobacilli accessible to genetic engineering.

**Phages.** *Lactobacillus* phages causing slower acidification in food fermentation deserve much interest because of their commercial importance (cf. Sharpe, 1981). The morphology of numerous double-stranded DNA phages virulent to many species has been described. Physicochemical parameters of seven phages are known, the data being summarized by Sozzi et al. (1981) who grouped the lactobacillus phages in accordance with the system of Bradley (1967) and Ackermann (1974). With the exception of one tailless phage from *L. plantarum*, all phages belong to group A or B and possess hexagonal heads and long contractile or noncontractile tails. They are basically similar to phages against other groups of bacteria.

Lysogeny is widespread within the genus. Yokokura et al. (1974) found that 40 strains belonging to seven species, out of a total of 148 strains belonging to 15 different species were lysed with mitomycin C. Thirty-one of 40 lysates showed phage-like particles by electron microscopy. Some of these particles produced plaques while others were defective phages, unable to produce plaques. Stetter (1977) found that 17 out of 21 strains of streptobacteria were lysogenic when induced with mitomycin C. Two of these phages were homoimmune with the *L. casei* phage PL1, which showed a surprisingly narrow host range (Stetter et al., 1978). Thus, it is suggested that frequent lysogeny caused by homoimmune phages may be responsible for the very narrow host ranges of lactobacillus phages. It may also explain why attempts to initiate phage typing schemes were not successful (Coetzee et al., 1980).

**Bacteriocins.** Bacteriocinogenic strains have been found among homo- and heterofermentative species (cf. Tagg et al., 1976; cf. Konisky, 1978). Early papers on bacteriocins, especially those from *L. acidophilus*, reported a very broad activity spectrum. Thus it is questionable whether these substances represent true bacteriocins (Barefoot and Klaenhammer, 1983). Lactocin B, a well-defined bacteriocin recently isolated from *L. acidophilus*, has a very narrow activity spectrum, restricted to only a few homofermentative species related to *L. acidophilus* (Barefoot and Klaenhammer, 1983). Also, the bacteriocins isolated from *L. fermentum* (DeKlerk and Smit, 1967) and lactocin LP27 from *L. helveticus* (Upreti and Hunsdill, 1973, 1975) are only active against lactobacilli. Bacteriocin typing of a large number of strains (Filippov, 1976a, b; Filippov and Rubanenko, 1977) showed a fairly wide range of sensitive species on the one hand, but also led to a subdivision of many species into various types. This indicates that bacteriocin typing may be more useful to characterize specific strains rather than to identify species.

**Antigenic structure.** Many strains of lactobacilli can be assigned to seven serological groups based on specific antigenic determinants (cf. Sharpe, 1970, 1981, Table 14.2). Groups A, D, F and G are specific for *L. helveticus*, *L. plantarum*, *L. fermentum* and *L. salivarius*, respectively. A few strains belonging to *L. plantarum* according to phenotypical characteristics could not be assigned to group D. They do not contain ribitol teichoic acid, the typical D antigen, but an unusual glycerol teichoic acid (Adams et al., 1969; Archibald and Couper, 1971). The chemical nature of the antigen of group G, an acid released polysaccharide with rhamnose as determinant, was recently studied by Knox et al. (1980).

Most strains of *L. casei* belong either to group B or C. However, strains of *L. casei* subsp. *rhamnosus* belong exclusively to group C. They possess a capsular, rhamnose-containing typing antigen. Its quantity is dependent on the cultural conditions (Wicken et al., 1983).

The homofermentative species *L. delbrueckii* and the two heterofermentative species *L. brevis* and *L. buchneri* belong to group E. The common antigen of these taxonomically distant species is a cell wall glycerol teichoic acid (Knox and Wicken, 1973, 1976).

An alternative serological nomenclature was proposed by Shimohashi and Mutai (1977). However, their scheme is based on a complex array of chemically undefined components and has thus no advantage over

Table 14.2.  
Group antigens of lactobacilli\*

Species	Group	Antigen	Location	Determinant
<i>L. helveticus</i>	A	GTA	Wall mem-brane	$\alpha$ -Glc
<i>L. casei</i>	B	Polysaccharide	Wall	$\alpha$ -Rha
<i>L. casei</i>	C	Polysaccharide	Wall	$\alpha$ -Glc
<i>L. plantarum</i>	D	RTA	Wall	$\alpha$ -Glc
<i>L. delbrueckii</i>	E	GTA	Wall	
subsp. <i>lactis</i>				
subsp. <i>bulgaricus</i>				
<i>L. brevis</i>	E	GTA	Wall	
<i>L. buchneri</i>	E	GTA	Wall	
<i>L. fermentum</i>	F	GTA	Membrane	$\alpha$ -Gal
<i>L. salivarius</i>	G	Polysaccharide	Wall	Rha

\* Symbols: GTA, glycerol teichoic acid; RTA, ribitol teichoic acid; Glc, D-glucosyl; Rha, L-rhamnosyl; Gal, D-galactosyl. (After Sharpe, 1981.)

the nomenclature developed by Sharpe (1955) which is used in Table 14.2.

**Antibiotic and drug sensitivity.** Lactobacilli are sensitive toward most antibiotics active against Gram-positive bacteria (Sutter and Finegold, 1976). *L. delbrueckii* subsp. *bulgaricus* is often used to detect antibiotics in milk.

Studies on the sensitivity or resistance pattern of lactobacilli towards antibiotics originated mainly from problems created by the presence of antibiotics in milk derived from mastitis therapy (Marth and Erickson, 1974; Sozzi and Smiley, 1980).

The sensitivity of intestinal lactobacilli toward antibiotics employed as feed additives has also been studied (Dutta and Devriese, 1981). Bile resistance was thought to be important for colonizing the intestine with lactobacilli. Therefore it was mainly studied in *L. acidophilus* (Kleenhammer and Kleeman, 1981).

Production of antibiotic substances by lactobacilli has repeatedly been claimed (Schroeder et al., 1980; Lindgren and Clevström, 1978a,b; DeKlerk and Coetzee, 1961). However, frequently there is no clear distinction between an antibiotic effect and the inhibition effects of lactic acid and/or H<sub>2</sub>O<sub>2</sub> produced by the organism. No defined and commercially used antibiotic from lactobacilli is yet known.

**Pathogenicity.** Apart from dental caries (Rogosa et al., 1953), lactobacilli are generally considered to be apathogenic. However, there is an increasing number of reports that lactobacilli have been involved in human diseases (Sharpe et al., 1973a; Berger, 1974; Bayer et al., 1978; Bourne et al., 1978). Mainly *L. casei* subsp. *rhamnosus*, but also *L. acidophilus*, *L. plantarum* and occasionally *L. salivarius* have been found to be associated with subacute bacterial endocarditis, systemic septicemia and abscesses. In a recent study, a homofermentative lactobacillus was the only organism isolated in pure culture from a case of chorioamnionitis (Lorenz et al., 1982), and *L. gasteri* was found in a case of urosepsis (Dickless et al., 1984). The many cases in which lactobacilli have been isolated from diseased tissue indicate their potential pathogenicity. However, the biochemical basis of such pathogenicity is as yet unknown. The finding that some rumen lactobacilli decarboxylate indoleacetic acid to skatol, a compound known to be responsible for acute bovine pulmonary emphysema, the naturally occurring form of the bovine respiratory disease (Yokoyama and Carlson, 1981), may be a first positive step in elucidating the pathogenicity of lactobacilli.

**Ecology, habitats and biotechnology.** Lactobacilli grow under anaerobic conditions or at least under reduced oxygen tension in all habitats providing ample carbohydrates, breakdown products of protein and nucleic acids, and vitamins. A mesophilic to slightly thermophilic temperature range is favorable. However, strains of some species (e.g.

*L. viridescens*, *L. sake*, *L. curvatus*, *L. plantarum*) grow—although slowly—even at low temperatures close to freezing point (e.g. refrigerated meat (Kitchell and Shaw, 1975), fish (Schroeder et al., 1980). Lactobacilli are generally aciduric or acidophilic. They decrease the pH of their substrate by lactic acid formation to below 4.0, thus preventing, or at least severely delaying, growth of virtually all other competitors except other lactic acid bacteria and yeasts. These properties make lactobacilli valuable inhabitants of the intestinal tract of man and animals and important contributors to food technology.

Several individual species have adapted to specific ecological niches and are generally not found outside their specialized habitats. The relative ease with which such species can be reisolated from their respective sources since their first discovery, sometimes almost 100 years ago, indicates that these niches are, in fact, their natural habitats.

**Plant sources.** Lactobacilli occur in nature in low numbers at all plant surfaces (Keddie, 1959; Mundt and Hammer, 1968) and together with other lactic acid bacteria grow luxuriously in all decaying plant material, especially decaying fruits. Hence, lactobacilli are important for the production as well as the spoilage of fermented vegetable feed and food (e.g. silage, sauerkraut, mixed pickles) and beverages (e.g. beer, wine, juices). Species chiefly isolated have been: *L. plantarum*, *L. brevis*, *L. coryniformis*, *L. casei*, *L. curvatus*, *L. sake*, *L. fermentum* (cf. Carr et al., 1975; Sharpe, 1981; Steinhaus, 1983; Kandler, 1984).

Several species are typical of specific products. Thus, *L. delbrueckii* subsp. *delbrueckii* exhibiting a very narrow range of fermented carbohydrates is the characteristic thermophilic organism found in potato and grain mashes fermented at 40–55°C (Henneberg, 1903).

It is also employed in the fermentation of millet mash to produce Bantu beer (Novellie, 1968), and is used for industrial production of lactic acid from molasses (Buchta, 1983).

Another specifically adapted species is *L. sanfrancisco*, the dominant acid producer in Californian sour dough (Kline and Sugihara, 1977). The organism isolated from European sour dough, designated *L. brevis* var. "lindneri" by Spicher and Schroeder (1978) also proved to belong to the species *L. sanfrancisco* (Weiss, Schillinger and Spicher, personal communication). An organism specifically used for the production of mainly L(+)-lactic acid-containing sauerkraut is *L. bavaricus* (Stetter, 1974). *L. hilgardii* and *L. fructivorans* (Fornath et al., 1949) are typical organisms of acidic and alcoholic beverages. *L. collinoides* (Carr and Davis, 1972) and *L. jamaicensis* (Carr and Davis, 1970; Carr et al., 1977) are found in cider and other fruit juices.

Although many different species of lactobacilli have been found in spoiled beer (Rainbow, 1975; Kirsoy and Dolan, 1978), a very important lactobacillus in beer spoilage is probably "*L. lindneri*", a so far incompletely described species which requires the addition of beer to the medium for detection and isolation (Bäck, 1981). Among the slime-forming spoilage organisms in sugar factories (Tilbury, 1978), *L. confusus* is the most common species of lactobacilli (Sharpe et al., 1972), growing in sucrose concentrations up to 15%.

**Milk and dairy products.** Milk contains no lactobacilli when it leaves the udder, but becomes very easily contaminated with lactobacilli by dust, dairy utensils, etc. Since streptococci grow faster, the number of lactobacilli remains usually fairly low even in spontaneously soured milk. Only after prolonged incubation do lactobacilli take over, due to their higher acid tolerance. In sour whey, the most acid tolerant, and thus typical species, which produces as much as 3% lactic acid, is *L. helveticus*. It is traditionally used in starters for the production of Swiss cheese and other types of hard cheeses, e.g. Grana, Gorgonzola and Parmesan (Bottazzi et al., 1973). Nowadays *L. delbrueckii* subsp. *bulgaricus* or subsp. *lactis* are also used (Biede et al., 1976; Auclair and Accolas, 1983). In all types of cheese with ripening periods longer than about 14 days, several mesophilic lactobacilli (*L. plantarum*, *L. brevis*, *L. casei*, etc.) originating from the milk or the dairy environment, reach levels as high as 10<sup>6</sup>–10<sup>8</sup>/g cheese (Sharpe, 1962; Abo, Elmag and Kandler, 1985a; van Kerken and Kandler, 1986).

Very specifically adapted lactobacilli for the production of sour milks are *L. delbrueckii* subsp. *bulgaricus*, a component of the well-known yogurt flora (Davis, 1975), and *L. kefir* (Kandler and Kunath, 1983);



the heterofermentative component of the Caucasian sour milk kefir. These two sour milks are the only known habitats of these two lactobacilli.

Although several species of lactobacilli may contribute to spoilage of dairy products by slime or gas production, only two species cause specific spoilage. *L. malvaromicus* may be responsible for malty flavor in milk (Miller et al., 1974) and *L. bifementans* has been found to cause the blowing of Edam cheese (Pette and Van Beynum, 1943).

**Meat and meat products.** Lactobacilli play an important role in the curing process of fermented sausages containing added sucrose. The most common naturally occurring species found in ripening raw sausages are *L. plantarum*, *L. brevis*, *L. farciminius*, *L. alimentarius* and "atypical" lactobacilli (Reuter, 1970, 1975) recently identified as *L. sake* and *L. curvatus* (Kagermeier, 1981; Kagermeier et al., 1985). In addition to streptococci, pediococci and micrococci/staphylococci, starters added to the meat mix often contain *L. plantarum* (Bacus and Brown, 1981; Robinson, 1983; Liepe, 1983).

Various species of lactobacilli multiply during cold storage of meat products. This delays spoilage by proteolytic bacteria, but may also lead to spoilage by producing off-flavor, acid taste, gas, slime or greening (Egan, 1983). While *L. viridescens* has been shown to cause greening (Niven and Evans, 1957), the role of the other species frequently isolated from stored meat—*L. plantarum*, *L. brevis*, and unidentified lactobacilli—is not clear. Some of the "atypical" homofermentative lactobacilli described by Hitchener et al. (1982) have been identified as *L. sake* and *L. curvatus* (Kagermeier et al., 1984). The unidentified heterofermentative strains found by Hitchener et al. (1982), which are characterized by the production of L(+) lactic acid, may be identical with *L. divergens*, the recently described new species isolated from vacuum-packaged raw minced meat in South Africa (Holzapfel and Gerber, 1983).

**Fish and marinated fish.** Although lactobacilli have not been considered to be indigenous to the marine environment, Kraus (1961) and Schröder et al. (1980) have shown that herring caught far from populated areas and fish and krill from the arctic environment harbor cold-adapted lactobacilli resembling *L. plantarum*. However, one of these isolates, studied in more detail with respect to its ability to decarboxylate amino acids (Jonsson et al., 1983), forms exclusively L(+) lactic acid, indicating that it represents a new, so far undescribed cryophilic species rather than *L. plantarum*. Homo- and heterofermentative lactobacilli play an important role in the spoilage of raw marinated herring (Blood, 1975). It is suggested that the acetic acid added to the herring provides the necessary acid environment for the action of proteinases present in the fish muscle (Meyer, 1962). The free amino acids thus liberated then provide the energy source for acetic acid-tolerant and salt-tolerant lactobacilli which are able to decarboxylate amino acids. The CO<sub>2</sub> formed is the first indication of spoilage. In carbohydrate-containing marinades, the carbohydrates may be the source of CO<sub>2</sub> liberated by heterofermentative lactobacilli. Therefore, Meyer (1956) distinguished between a "carbohydrate" swell and a "protein" swell. Lactobacilli isolated from marinated herring were mainly allotted to *L. plantarum*, *L. brevis* and *L. buchneri*. However, reinvestigation of such isolates employing modern biochemical and genomic characteristics is necessary to elucidate their true taxonomic position. Unidentified lactobacilli have also been isolated from fresh water salmonides (Evelyn and McDermott, 1961) and diseased rainbow trout (Cone, 1982).

**Man and animals.** The intestinal tract of man and animals harbors many species of lactobacilli (Lerche and Reuter, 1962; Mitsuoka, 1969) living as commensals intimately associated with the mucous surface epithelium. This subject has been extensively reviewed (Savage, 1977; Sharpe, 1981). Only the few species found exclusively, or at least predominantly, in the intestinal tract will be discussed here.

*L. salivarius* may be the most typical species of the mouth flora, although it is also found in the intestinal tract (Rogosa et al., 1953). The other species found are much more universally distributed in nature.

The most prominent species, probably indigenous to the intestine, is *L. acidophilus*, which is believed to exert a beneficial effect on human

and animal health. It is used on an industrial scale in preparing acidophilus sour milk and producing pharmaceutical preparations (Rehm, 1983) for restoring the normal intestinal flora after disturbance caused by diseases or treatment with antibiotics. Whether such preparations contain true *L. acidophilus* strains, and which strains, if any, have a beneficial influence in the particular individual remains a controversial topic (Lauer et al., 1980). The problem is further complicated by the finding that strains designated as *L. acidophilus* proved to belong to many different genotypes exhibiting only a low degree of DNA-DNA homology with each other (Johnson et al., 1980; Sarra et al., 1980; Lauer et al., 1980). While most genotypes cannot be distinguished on the basis of phenetic characteristics, two genotypes could be phenotypically separated, and one of them has been described as the new species *L. gasseri* (Lauer and Kandler, 1980). Another recently described homofermentative species, *L. animalis* (Dent and Williams, 1982) also phenotypically resembling *L. acidophilus*, was detected in dental plaques of primates and in the intestine of dog and mouse. It could be separated from *L. acidophilus* mainly on the basis of the protein pattern obtained in electrophoresis and the formation of exclusively L(+) lactic acid.

Recently, strains belonging to an additional genotype of *L. acidophilus* or to the genotype IIB of *L. gasseri* were isolated from kefir. These strains represent the majority population of lactobacilli in the kefir grain, but only a minority in the final sour milk product, where the heterofermentative species *L. kefir* dominates (Kunath and Kandler, 1984). The distinct heterogeneity of the species *L. acidophilus* is a challenge to all intestinal microbiologists.

Among the heterofermentative intestinal lactobacilli, *L. fermentum* was considered to be the dominant species (Lerche and Reuter, 1962) in the intestine. A taxonomic study of several strains designated as *L. fermentum* based on the sugar fermentation pattern revealed that two groups of strains, representing two species exhibiting a G + C content of 53 mol% and 41 mol%, respectively, had been included together. Strains possessing the lower G + C value were described as the new species *L. reuteri* (Kandler et al., 1980), which includes most strains isolated from the intestine by Lerche and Reuter (1962). *L. reuteri* was also found to be the dominating heterofermentative species in the intestine of calves (Sarra et al., 1979). Thus *L. reuteri* may be the main heterofermentative lactobacillus species in the intestine, while *L. fermentum* seems to be more widespread in lactic acid fermented substrates. However, this suggestion needs further confirmation.

*L. murinus*, a recently described homofermentative species, has been isolated from the feces of mice and rats. It may be a typical species in the intestine of rodents (Haimé et al., 1980).

Lactobacilli are also found in the rumen of ruminants. However, they are rarely classified at the species level. Two anaerobic species, *L. ruminis* and *L. vitulinus*, have been described from the bovine rumen. *L. ruminis* has also been isolated from the human intestine (Sharpe et al., 1973b).

**Sewage and manure.** Sewage and manure are secondary habitats of all lactobacilli found in the intestine, but also of some other species not, or only rarely, found in the intestine. In manure, *L. coryniformis* and *L. curvatus*, neither recorded as intestinal, are frequently found (Abo Elnaga and Kandler, 1985a). *L. vaccinostercus* has only been found in cow dung as yet (Okada et al., 1979).

In municipal sewage, levels of 10<sup>4</sup>–10<sup>6</sup> lactobacilli/ml have been found (Weiss et al., 1981). The heterofermentative strains (~25%) of the isolates have been classified as *L. fermentum*, *L. reuteri*, *L. brevis* and, to a lesser extent, as *L. confusus*. The homofermentative strains (~75%) of the isolates belonged to a larger number of different species. However, about 10% of the strains could not be allotted to any of the known species. They have been described as representatives of the two new species *L. sharpeae* and *L. agilis*, not as yet found in any other habitat (Weiss et al., 1981).

#### Enrichment and Isolation Procedure

Procedures for the isolation of lactobacilli must take into account their aciduric or acidophilic nature, their complex nutritional require-

ments and their preference for microaerophilic conditions. When lactobacilli are the predominant flora in the source material, the rather nonselective MRS\* agar (de Man, Rogosa and Sharpe, 1960) or the somewhat similar APT agar (Evans and Niven, 1951) may be used for isolation. APT agar is commonly used for isolating *L. viridescens* and other lactobacilli from meat products. When lactobacilli occur only as part of a complex population, selective media are required. Most lactobacilli from many different sources have been successfully isolated on the widely used acetate medium† (SL) of Rogosa, Mitchell and Wiseman (1951). However, SL medium is not completely selective for lactobacilli as other lactic acid bacteria, e.g. leuconostocs, pediococci, enterococci, bifidobacteria (intestinal sources) and yeasts may also grow. Thus, colonies may have to be further examined. Yeasts may be eliminated by the addition of cycloheximide at a concentration of 100 mg/liter.

On the other hand, some lactobacilli, mainly from quite specialized environments, will not grow on SL medium. Depending on the source of isolation, minor modifications of SL medium, supplementing it with more or less specific growth factors such as meat extract, tomato juice, fresh yeast extract, malt extract, ethanol, mevalonic acid (sake) or even some of the natural substrate (beer, different juices) can improve the isolation of lactobacilli which are highly adapted to the conditions of their ecological niches. Replacement of glucose, either completely or partially, by other carbohydrates such as maltose, fructose, sucrose or arabinose is recommended in some cases, especially where heterofermentative lactobacilli play an important role. For the detection of beer-spoiling bacteria including nutritionally fastidious lactobacilli, a special medium (NBB medium) has been described by Back (1980). For further information reference is given to Sharpe (1981) where many media and methods of cultivating lactobacilli are compiled in detail.

For the isolation of anaerobic lactobacilli from intestinal sources 0.05% (w/v) cysteine should be added and it may be necessary to pre-reduce poured, dried plates by overnight incubation in an anaerobic jar.

Since most lactobacilli generally grow better either anaerobically or in the presence of increased CO<sub>2</sub> tension, agar plates should be incubated in jars evacuated and filled with 90% N<sub>2</sub> or H<sub>2</sub> + 10% CO<sub>2</sub> or in anaerobic jars (BBL, Oxoid) using H<sub>2</sub> + CO<sub>2</sub> generating kits.

#### Maintenance Procedures

For short-term preservation, cultures are preferably inoculated into MRS or optimal medium agar slabs, incubated until growth becomes visible, stored at 4–7°C and transferred monthly. Some species or strains, however, die out quite rapidly within a series of transfers. Alternatively, cultures grown to the early stationary growth phase may be deep frozen in the growth medium and stored at –20°C for several months.

The method of choice for long-term preservation is lyophilization. Cells grown to the late logarithmic growth phase are collected by centrifugation, resuspended in sterile skim milk or horse serum containing 7.5% (w/v) glucose and lyophilized. Ampules are sealed under vacuum and stored at 5–8°C. Most strains preserved by this method are still viable after 10–20 years, although some require more frequent re-lyophilization. Strains may also be kept for long periods (over 30 years) in liquid nitrogen.

#### Procedure for Testing Special Characters

**Carbohydrate fermentation.** MRS broth without meat extract and glucose with 0.05% (w/v) chlorophenol red is generally used as basal

medium. Filter-sterilized solutions of the test carbohydrates are added to a final concentration of 1%. Tests are incubated at the optimum growth temperature and results recorded up to 7 days. In a few cases, e.g. some strains of *L. delbrueckii*, the addition of 0.2% meat extract broadens the pattern of fermented carbohydrates somewhat and the fermentation of glucose is distinctly improved. For strains which will not grow reasonably in MRS broth the optimal growth medium should be used as basal medium.

**Lactic acid configuration.** The amount of the isomers of lactic acid produced is best determined enzymatically using D-lactate (Gawehn and Bergmeyer, 1974) and L-lactate dehydrogenase (Gutmann and Wahlefeld, 1974).

Corrections must be made for the lactic acid content of the medium before inoculation. Care must be taken to analyze cultures after they have reached the stationary growth phase, since some DL-formers produce predominantly L(+) or, in a few cases, D(–) lactic acid during the early growth phase.

**Cell wall analysis.** The absence or presence of meso- or LL-diaminopimelic acid (meso-DAP; LL-DAP) in the cell wall may be tested by the following simple procedure: cells from about 1 ml of broth culture or a loopful of cell material taken from an agar plate or a slant are hydrolyzed with 0.5 ml 6 M HCl at 100°C, overnight, in a sealed ampule. HCl is removed by a gentle stream of air on the hydrolysate at about 50°C; the residue is taken up in a minimum of water, applied to a thin layer plate (precoated cellulose plastic sheets are recommended), developed in the solvent system: methanol:pyridine:water:10 M HCl (320:40:70:10 v/v/v/v) for 2–3 hours and sprayed with acidic ninhydrin; meso- and LL-DAP are well separated from all other amino acids due to their very low R<sub>f</sub> value. They are further characterized by their olive green color which changes to yellow after several hours or days in the dark.

For details of the peptidoglycan composition, purified cell walls must be prepared. In most cases the rapid screening method, e.g. boiling the washed cells with trichloroacetic acid followed by digestion with trypsin (Schleifer and Kandler, 1972), is satisfactory. Lysine and ornithine can be distinguished by the chromatographic method described above. It is the least time-consuming test to differentiate *L. reuteri* from *L. fermentum*.

The peptidoglycan-type Lys-DAsp, most widely distributed within the genus *Lactobacillus*, is well characterized by the occurrence of N<sup>ε</sup>-(aminosuccinyl)-lysine, a derivative of N<sup>ε</sup>-(aspartyl)-lysine formed during acid hydrolysis of cell walls (4N HCl, 100°C, 16 hours). It can be easily detected by two-dimensional paper chromatography (first direction: isopropanol:acetic acid:water 75:10:15; second direction: α-picoline:25% NH<sub>4</sub>OH:water 70:2:28). Other peptidoglycan types may be analyzed by the methods described in detail by Schleifer and Kandler (1972).

Teichoic acids may be extracted from cell walls with 70% hydrofluoric acid at 0°C and analyzed by liquid gas chromatography according to Fiedler et al. (1981).

**Characterization of lactic acid dehydrogenases.** The electrophoretic mobility of the lactic acid dehydrogenases (LDH) is determined by polyacrylamide gel electrophoresis at pH 7.5 using crude cell extracts according to Hensel et al. (1977). L-LDH rabbit Iso-I (Boehringer, Mannheim) serves as reference. Whether the L-LDH of an organism is allosteric or not is tested by spectrophotometric measurement of the rate of pyruvate reduction with and without the effectors fructose-1,6-diphosphate (FDP) and Mn<sup>2+</sup> at pH 6.5 in dialyzed crude cell extracts (Hensel et al., 1977).

\* MRS agar: casein peptone, 10.0 g; meat extract, 10.0 g; yeast extract, 5.0 g; glucose, 20.0 g; K<sub>2</sub>HPO<sub>4</sub>, 5.0 g; diammonium citrate, 2.0 g; Na acetate, 5.0 g; MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.5 g; MnSO<sub>4</sub>·4 H<sub>2</sub>O, 0.2 g; Tween 80, 1.0 g; agar, 15.0 g; distilled water 1000 ml; adjust pH to 6.2–6.4 and sterilize at 121°C for 15 min.

† Selective SL medium: casein peptone, 10.0 g; yeast extract, 5.0 g; KH<sub>2</sub>PO<sub>4</sub>, 6.0 g; diammonium citrate, 2.0 g; MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.5 g; MnSO<sub>4</sub>·4 H<sub>2</sub>O, 0.2 g; FeSO<sub>4</sub>·7 H<sub>2</sub>O, 0.04 g; Tween 80, 1.0 g; glucose, 20.0 g; Na acetate-3 H<sub>2</sub>O, 25.0 g; agar, 15.0 g; dissolve the agar separately by steaming in 500 ml distilled water; dissolve all the other ingredients without heating in 500 ml distilled water; adjust pH with glacial acetic acid to 5.4, then add this to the melted agar and boil for 5 min; no further sterilization is given.

### Differentiation from Other Closely Related Taxa

Lactobacilli are metabolically very similar to the other genera of the so-called lactic acid bacteria. Only their rod shape readily distinguishes them from the coccoid genera *Streptococcus*, *Leuconostoc* and *Pediococcus*. However, some species of the obligately heterofermentative lactobacilli form coccoid rods and may be confused with *Leuconostoc*. These species are differentiated from *Leuconostoc* by their formation of DL-lactic acid and not D(-)-lactic acid.

Strains of *Streptococcus* which form atypically elongated cells may also be confused with coccoid rods of lactobacilli. Here, differentiation may require nucleic acid hybridization as in the case of *L. xylophilus* and "*L. hordniae*," both of which have been shown to belong to the genus *Streptococcus* (Garvis et al., 1981; Kilpper-Bälz et al., 1982).

The rod-shaped bifidobacteria, which until the eighth edition of *Bergey's Manual* had long been included in the genus *Lactobacillus* as "*Lactobacillus bifidus*," may be differentiated from lactobacilli on the basis of their characteristic hexose fermentation pathway which yields lactic acid and acetic acid at a molar ratio of 2:3, but no CO<sub>2</sub>, instead of lactic acid, acetic acid (or ethanol) and CO<sub>2</sub> at a molar ratio of 1:1:1, the pattern of fermentation products typical of obligately heterofermentative lactobacilli.

### Taxonomic Comments

The species of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* form a supercluster within the so-called clostridia subbranch of the Gram-positive bacteria, as shown by oligonucleotide

cataloging of their 16S rRNA (Fig. 14.4; Stackebrandt et al., 1983). Bifidobacteria, already excluded from the family *Lactobacillaceae* in *Bergey's Manual*, eighth edition, have proved to be completely unrelated to lactobacilli. They belong to the so-called actinomycetales subbranch of the Gram-positive bacteria.

The neighborhood of the lactobacillus supercluster and the streptococcus cluster, and their position at the clostridia subbranch which also contains the aerobic bacilli (Fig. 14.4) is in accordance with Orla-Jensen's concept of "lactic acid bacteria" as a group of closely related microaerophilic genera. However, there is only limited agreement between the results obtained by oligonucleotide cataloging and the phylogenetic implications of serological studies involving antisera against malic enzymes (London, 1971), fructose-1,6-diphosphate aldolases (London and Kline, 1973; London and Chace, 1976) and glyceraldehyde-3-phosphate dehydrogenases (London and Chace, 1983) of various lactic acid bacteria and some anaerobic and aerobic bacteria. On the basis of the two techniques, only the very close interrelationship between the four genera of lactic acid bacteria and their origin from a common progenitor is certain. Different results were obtained not only regarding the relationship between the lactic acid bacteria and other phylogenetically more distant genera (*Eubacterium*, *Propionibacterium*, *Brochothrix*, *Acholeplasma*, *Aerococcus*) but also regarding the relationship within the lactic acid bacteria. The immunological grouping indicates a close relationship between streptococci and the *L. casei* group (London and Chace, 1983), whereas, on the basis of the 16S rRNA cataloging, only representatives of the genus *Streptococcus*, but not members of the genera *Pediococcus* and *Leuconostoc*, can be separated

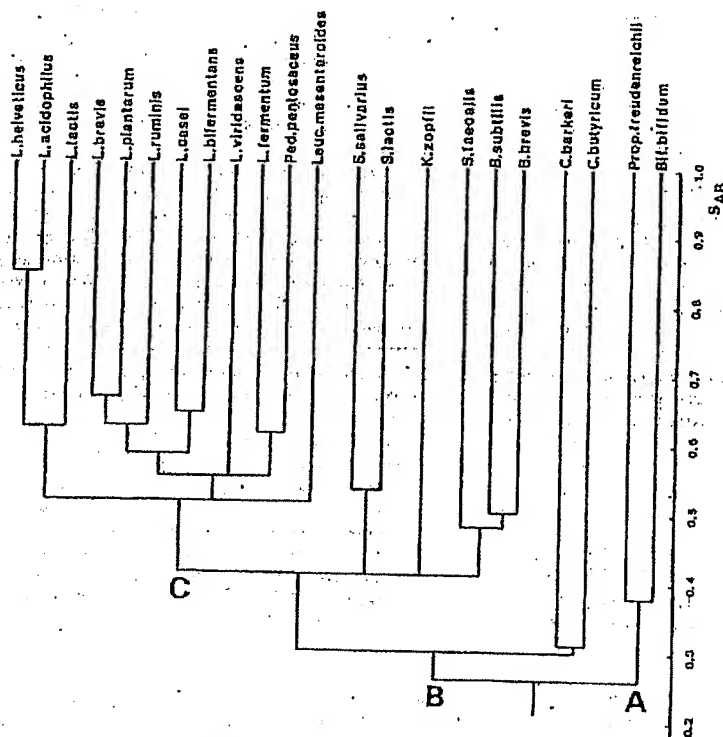


Figure 14.4. Dendrogram of relationship among representatives of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Kurthia*, *Clostridium*, *Propionibacterium*, *Bifidobacterium* and *Bacillus* based on SAB values (16S rRNA cataloging; Stackebrandt et al., 1983). A, actinomycetales subbranch; B, clostridia subbranch; and C, lactobacillus supercluster.

from the genus *Lactobacillus*. No subdivision of the genus *Lactobacillus* into three groups, corresponding to Orla-Jensen's genera "*Thermobacterium*," "*Streptobacterium*" and "*Betabacterium*," often referred to as subgenera (Sharpe, 1981), is indicated in the dendrogram based on  $S_{AB}$  values (Fig. 14.4). With the exception of the pair *L. helveticus* and *L. acidophilus*, which is related at the very high level of  $S_{AB} = 0.83$ , all investigated species exhibit low  $S_{AB}$  values between 0.47 and 0.65 indicating a considerable phylogenetic depth for each of the phenotypes. In addition, the small differences between the  $S_{AB}$  values suggest extensive speciation within a relatively short period of time, probably at the global "Pasteur point" when microaerophilic life became possible (Stackebrandt et al., 1983).

The high phylogenetic age of the genus *Lactobacillus* is also reflected by the wide range of the G + C content of DNA from 32–53 mol%—a span twice as large as is usually accepted for a single genus (cf. Schleifer and Stackebrandt, 1983), the lack of significant DNA/DNA homology between most of the species and the relatively high rate of amino acid exchange among pairs of *Lactobacillus* species in the highly conserved substrate-binding region of L-lactic acid dehydrogenase (Hensel et al., 1981; Mayr et al., 1982).

More work is needed to elucidate the phylogenetic structure of the genus *Lactobacillus* and the other genera constituting the "lactic acid bacteria." Hence, we shall not at present follow the suggestion of Stackebrandt et al. (1983) to expand the description of the genus *Lactobacillus* so as to comprise also the genera *Leuconostoc* and *Pediococcus*.

We shall arrange the species of *Lactobacillus* into the traditional three groups resembling Orla-Jensen's three genera without designating them as formal subgeneric taxa since they do not represent phylogenetically defined clusters. Although the majority of strains of each of the new groups agree with the original definition of thermobacteria, streptobacteria and betabacteria, many of the recently described species do not fit these definitions. Hence, the following new definitions contain neither growth temperature nor morphology, the classical characteristics of Orla-Jensen's subgenera.

Group I, obligately homofermentative lactobacilli: hexoses are fermented almost exclusively to lactic acid by the Embden-Meyerhof pathway; pentoses or gluconate are not fermented. Rare reports on pentose fermentation by particular strains of members of group I should be reinvestigated. Fermentation balances should be determined, in order to get information on the possible fermentation mechanism of such atypical strains. In a few cases, we have obtained strains claimed to ferment pentoses. However, they either did not ferment pentoses in our hands or did not belong to a species of group I.

Group II, facultatively heterofermentative lactobacilli: hexoses are fermented almost exclusively to lactic acid by the Embden-Meyerhof pathway or, at least by some species, to lactic acid, acetic acid, ethanol and formic acid under glucose limitation; pentoses are fermented to lactic acid and acetic acid via an inducible phosphoketolase.

Group III, obligately heterofermentative lactobacilli: hexoses are fermented to lactic acid, acetic acid (ethanol) and  $\text{CO}_2$ ; pentoses are fermented to lactic acid and acetic acid. In general, both pathways involve phosphoketolase. However, some species which probably possess other pathways for carbohydrate breakdown but performing also a heterofermentation including the production of gas from hexoses are tentatively also included in group III, e.g. *L. bifementans*.

Group I harbors all the classical representatives of Orla-Jensen's thermobacteria and many recently described species. With regard to DNA/DNA homology, group I contains two complexes of related species or subspecies and many single species not related to any significant extent on the basis of present knowledge. One of the two complexes consists of the three subspecies of *L. delbrueckii*. The type strains of the four former species, *L. delbrueckii*, *L. bulgaricus*, *L. lactis* and *L. leichmannii*, were found to possess between each other more than 80% DNA/DNA homology (Weiss et al., 1983b) and the phenotypical differences are restricted to variations in the range of fermented carbo-

hydrates. Thus they have been considered to justify only the rank of subspecies. *L. delbrueckii* subsp. *lactis* exhibits the widest range of fermented carbohydrates and may be the common ancestor from which several variants, adapted to specialized niches (sour milk, grain mashes, etc.) have evolved by only minor changes of the phenotype and genotype.

The second complex is represented by *L. acidophilus* which was shown to exhibit a distinct genomic heterogeneity. A large number of strains designated originally *L. acidophilus* has been arranged in two main groups of genotypes each consisting of several subgroups based on DNA/DNA homology (Johnson et al., 1980; Lauer et al., 1980; Sarra et al., 1980). DNA/DNA homology is 75–100% between strains of the same subgroup, 25–50% between strains of different subgroups within each of the two main groups and below 25% between strains of the two main groups. The two main groups exhibit clear phenotypic differences and are thus considered to represent two different species. The main group containing the original type strain of the species retains the name *L. acidophilus*, while the other group has been described as the new species *L. gasseri* (Lauer and Kandler, 1980). Recently, the type strain of the earlier described species *L. crispatus* (Moore and Holdeman, 1970) was found to be 100% homologous with one of the subgroups of *L. acidophilus* (Cato et al., 1983).

*L. helveticus* may be considered a highly specialized derivative of the *L. acidophilus* complex, adapted to sour whey. It resembles *L. acidophilus* with respect to the G + C content of DNA and many biochemical characteristics and possesses DNA/DNA homology of 19–44% with representatives of the various genotypes of *L. acidophilus* (Johnson et al., 1980). It shares also a high  $S_{AB}$  value with the type strain of *L. acidophilus* (Fig. 14.4; Stackebrandt et al., 1983). Thus, *L. acidophilus*, *L. gasseri*, *L. crispatus* and *L. helveticus* form a cluster of closely related species within group I, which is only distantly related to the *L. delbrueckii* complex ( $S_{AB} = 0.6$ ), represented by *L. delbrueckii* subsp. *lactis* in Figure 14.4.

Group II contains Orla-Jensen's streptobacteria and many newly described species. Three complexes of species or subspecies can be recognized, while the other species show no known phylogenetic relationship with each other.

One complex is formed by the strains designated *L. plantarum*. The phenotypical variation within this giant species have long been recognized. Strains exhibiting characteristics atypical for the genus *Lactobacillus*, e.g. motility, nitrate reduction, pseudocatalase, etc., have often been designated *L. plantarum*. A genomic heterogeneity of *L. plantarum* has been shown by DNA/DNA homology studies (Dellaglio et al., 1975). Although most of the strains investigated were related to the type strain at a homology level of 80–100%, a quarter of the strains was only related at a level of 30–70%. Three strains were highly related with a strain designated "*L. pentosus*" (Fréd. et al., 1971), a name considered to be synonymous with *L. plantarum* at present, but which may be revived in the future. Four other strains exhibited 57–70% DNA/DNA homology between each other and to the type strain of *L. plantarum*, thus indicating the existence of additional genotypes of *L. plantarum*.

A second complex of at least three genotypes is formed by the subspecies of *L. casei*. While the type strain and only two strains originally designated "*L. zeae*" (Kuznetsov, 1959) are related at a DNA/DNA homology level of 80–100%, the majority of the strains of *L. casei* subsp. *casei*, *L. casei* subsp. *pseudoplantarum* and *L. casei* subsp. *tolerans* form a second genotype at a homology level of 80–100% among each other, but with only 40% homology toward the genotype which contains the type strain. Strains of *L. casei* subsp. *rhamnosus* represent a third genotype which shares only 30–50% homology with strains of the other two genotypes. Because of the low DNA/DNA homology, and distinct phenetic differences to other subspecies (see Tables 14.7 and 14.8), *L. casei* subsp. *rhamnosus* is a candidate to be raised to the species status. The two other subspecies, although closely related to *L. casei* subsp. *casei* are phenotypically distinctly different by forming DL-lactic acid



via a lactic acid racemase (*L. casei* subsp. *pseudopiantarum*; Stetter and Kandler, 1973) or by heat tolerance and an extremely sparse pattern of fermented carbohydrates (*L. casei* subsp. *tolerans*; see Tables 14.7 and 14.8), respectively. *L. casei* subsp. *tolerans* does not ferment ribose and gluconate and therefore does not fit the definition of group II. However, the high DNA/DNA homology with *L. casei* indicates that the lack of these characteristics is caused by minor genomic differences.

The third complex of species consists of *L. sake*, *L. curvatus* and *L. bavaricus*. The first two species are related at a DNA/DNA homology level of 50%. Both species are characterized by possessing inducible lactic acid racemase which converts the primarily formed L-(+)-lactic acid to racemate (Stetter and Kandler, 1973). *L. bavaricus* (Stetter and Stetter, 1980) is phenotypically clearly different from the two species, by the lack of lactic acid racemase, but otherwise it is very similar. In fact, the type strain and most of the strains of *L. bavaricus* exhibit 100% DNA/DNA homology to *L. sake*, while a few strains are completely homologous with *L. curvatus* (Kagermeier et al., 1985). Thus *L. bavaricus* consists of two genotypes, one of which, including the type strain, may be considered as a subspecies of *L. sake*, the other as a subspecies of *L. curvatus*.

*L. casei*, *L. curvatus*, *L. sake* and *L. bavaricus* possess an allosteric L-lactic acid dehydrogenase with fructose-1,6-diphosphate and  $Mn^{2+}$  acting as effectors (Hensel et al., 1977). The properties of the L-lactic acid dehydrogenase enzymes of the various species are very similar. They show partial serological cross-reactions (Hensel, 1977) and their subunits form hybrids (Mayr et al., 1980). This could indicate a close phylogenetic relationship between these species. However, no significant DNA/DNA homology could be detected between *L. casei* and the other species. No  $S_{AB}$  values of these species are known as yet. An allosteric L-lactic acid dehydrogenase has also been found in *L. mirinus*. However, this enzyme has not been studied in detail. None of the other species of group II show a significant DNA/DNA homology to any other species or possess phenotypic characters which would indicate a specific relationship between any pair of strains.

Group III contains all the obligately heterofermentative gas-forming lactobacilli of Orla-Jensen's genus "*Betabacterium*" and several more recently described species. Two species—*L. biferrmentans* and *L. divergens*—which also form gas from glucose, probably do not possess the 6-phosphogluconate pathway. *L. biferrmentans* ferments glucose homofermentatively to DL-lactic acid, but—depending on pH—the lactic acid formed is more or less completely split into acetic acid,  $CO_2$  and  $H_2$  (Pette and van Beynum, 1943; Kandler et al., 1983). Although the formation of  $H_2$  is a characteristic not included in the description of the genus *Lactobacillus*, the organism is kept in this genus because of its distinct relationship to lactobacilli as evidenced by the dendrogram based on 16S rRNA cataloging (Fig. 14.4) and, more recently, by rRNA/DNA hybridization (Schillinger, unpublished). Considering the  $S_{AB}$  values and the rRNA/DNA hybridization data, *L. biferrmentans* is closely related to *L. casei*, an organism able to form acetic acid, ethanol and formic acid instead of lactic acid when grown under glucose limitation. However, *L. casei* does not possess a dehydrogenase for  $H_2$ .

evolution. It is tempting to suggest that *L. biferrmentans* is derived from *L. casei* by evolving a formate hydrogen ligase.

The fermentation balance of *L. divergens* indicates that this organism also does not possess the 6-phosphogluconate pathway. On a molar basis, the proportion of the  $C_2$  compounds (acetic acid and ethanol) and  $CO_2$  formed from hexoses is too small compared to that of lactic acid. Also pentose fermentation does not yield the proper molar ratios of fermentation products. Here, the  $C_2$  compounds are favored compared to lactic acid. Although the details of the fermentation mechanism remain to be elucidated, *L. divergens* is clearly heterofermentative and thus included in group III.

Most species of group III fall within a very narrow range of G + C content of DNA (40–46 mol%). However, they do not show significant DNA/DNA homology between each other (Vescovo et al., 1979). Except the pair *L. kefir* and *L. buchneri*, exhibiting a DNA/DNA homology of 40% (Kandler and Kunath, 1983), no reliable clustering of species within group III is possible. However, it is suggested that species in which the Lys-Asp type peptidoglycan—most common among lactobacilli—is replaced by the Lys-Ala-Ser or chemically similar types—typical of the genus *Leuconostoc*—may form a cluster related to *Leuconostoc*. Such a relationship has already been suggested in the case of *L. viridescens* and *L. confusus* (Garvie, 1975), species which contain Lys-Ser-Ala and Lys-Ala type peptidoglycan, respectively. On the other hand, members of the two genera are sometimes confused. Thus, *L. confusus* was originally allotted to *Leuconostoc* because of its coccoid appearance and slime formation, whereas the heterofermentative, coccoid, "*L. batatas*" (ATCC 15520), described by Kitahara (1949), was found to form D(-)-lactic acid and belongs to *Leuconostoc* (Weiss, unpublished).

The phylogenetic structure of group III needs further elucidation with the help of DNA/RNA hybridization and the determination of  $S_{AB}$  values of a greater number of species.

#### Acknowledgment

We are indebted to the ATCC, DSM, NCIB, NCDO and VPI for supplying numerous strains. We owe many ideas and information to the stimulating discussions we have had over years with our friends from the subcommittee of lactobacilli and related organisms.

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### Differentiation and characteristics of the species of the genus *Lactobacillus*

The differential characteristics of the species of *Lactobacillus* are indicated in Tables 14.3 and 14.4. Other characteristics of the species

are listed in Tables 14.5–14.10.

#### List of the species of the genus *Lactobacillus*

1. *Lactobacillus delbrueckii* (Leichmann 1896) Beijerinck 1901, 229<sup>11</sup> (*Bacillus Delbrückii* (sic) Leichmann 1896, 284.)

Note. Because of the high phenotypic and genomic similarities between *L. delbrueckii*, *L. leichmannii*, *L. lactis* and *L. bulgaricus* only *L. delbrueckii* is here retained as a separate species, whereas both *L. lactis* and *L. leichmannii* are treated as *L. delbrueckii* subsp. *lactis* and *L.*

*bulgaricus* as *L. delbrueckii* subsp. *bulgaricus* (see Weiss et al., 1983b, 1984).

*delbrueckii* M.L. gen. n. *delbrueckii* of Delbrück, named for M. Delbrück, a German bacteriologist.

Rods with rounded ends, 0.5–0.8 by about 2–9  $\mu$ m, occurring singly and in short chains.

Table 14.3.

Differential characteristics of the obligately homofermentative and facultatively heterofermentative species of the genus *Lactobacillus*\*

Species	Mol% G + C	Teichoic acid	Starch	Meli- biose	Man- nose	Man- nitol	Maltose	Sucrose	D(-)- Lac- tic acid	L(+)- Lac- tic acid	DL- Lac- tic acid	Growth at 15°C	Ribose	mDpm in pep- tido- glycan
25. <i>L. plantarum</i>	45	+									+	+	+	+
23. <i>L. mollaromicus</i>	36	-								+				
16. <i>L. ogilis</i>	44	-								+				
13. <i>L. sharpeae</i>	53	+					+	-		+		+	-	
15. <i>L. yamianashiensis</i>	33	-					-	+						
11. <i>L. ruminis</i>	44	-								+				
14. <i>L. vitulinus</i>	35	-							+					
24. <i>L. murinus</i>	43	-						+		+			+	-
22. <i>L. homophilochii</i>	36	+				-		-			+	+		
19b. <i>L. casei</i> subsp. <i>pseudoplantarum</i>	46	-				+		+						
26. <i>L. sake</i>	43	-		+		-								
21. <i>L. curvatus</i>	43	-		-										
18. <i>L. bacaricus</i>	43	-				-				+				
19a. <i>L. casei</i>	46	-				+								
17. <i>L. alimentarius</i>	36	-				-								
20. <i>L. coryniformis</i>	45	-				+			+			+	-	
3. <i>L. amylophilus</i>	45	-	+			-				+				
7. <i>L. farciminis</i>	35	-	-											
5. <i>L. animalis</i>	42	-			+					+				
12. <i>L. salivarius</i>	35	-			-									
9. <i>L. helveticus</i>	39	+						-			+			
4. <i>L. amyloovorus</i>	44	+	+					+						
2. <i>L. acidophilus</i>	36													
6. <i>L. crispatus</i>	36													
8. <i>L. gasserii</i>	34	-												
1. <i>L. delbrueckii</i>	50	+							+					
10. <i>L. jensenii</i>	35													

\* Symbols: +, 90% or more of the strains are positive; -, 90% or more of the strains are negative.

Good growth at 45°C or even at 48–52°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

Growth factor requirements: pantothenic acid and niacin generally essential; riboflavin, folic acid, vitamin B<sub>12</sub> and thymidine are essential for particular strains; thiamine, pyridoxin, biotin and p-aminobenzoic acid are not required.DNA/DNA homology: strains labeled *L. delbrueckii*, *L. bulgaricus*, *L. lactis* and *L. leichmannii*, including the respective type strains, were found highly homologous among each other (Weiss et al., 1983b); no genomic relationship could be detected to *L. helveticus* (Simonds et al., 1971; Dellaglio et al., 1973).The mol% G + C of the DNA is 49–51 (Bd, T<sub>m</sub>).

Three subspecies are presently recognized.

1a. *Lactobacillus delbrueckii* subsp. *delbrueckii* (Leichmann 1896) Weiss, Schillinger and Kandler 1984, 270.<sup>VP</sup> (Effective publication: Weiss, Schillinger and Kandler 1983b, 556.)

Distinguishing characteristics are given in Tables 14.5 and 14.6.

Isolated mainly from plant material fermented at high temperatures (40–53°C).

Type strain: ATCC 9649.

1b. *Lactobacillus delbrueckii* subsp. *bulgaricus* (Orla-Jensen 1919) Weiss, Schillinger and Kandler 1984, 270.<sup>VP</sup> (Effective publication: Weiss, Schillinger and Kandler 1983b, 556.) (*Thermobacterium**bulgaricum* Orla-Jensen 1919, 164; *Lactobacillus bulgaricus* Rogosa and Hansen 1971, 181.)*bulgaricus* M.L. adj. *bulgaricus* Bulgarian.

Ferments only a few carbohydrates as shown in Table 14.5. n-LDH migrates distinctly faster in electrophoresis than that of the other subspecies.

Isolated from yoghurt and cheese.

Type strain: ATCC 11842 (DSM 20081).

1c. *Lactobacillus delbrueckii* subsp. *lactis* (Orla-Jensen 1919) Weiss, Schillinger and Kandler 1984, 270.<sup>VP</sup> (Effective publication: Weiss, Schillinger and Kandler 1983b, 556.) (*Thermobacterium lactis* Orla-Jensen 1919, 164; *Lactobacillus leichmannii* (Henneberg) Borgey et al. 1923, 249.)*lactis*, L. n. *lac* milk; L. gen. n. *lactis* of milk.

Distinguishing characteristics are given in Tables 14.5 and 14.6.

Isolated from milk, cheese, compressed yeast and grain mash.

Type strain: ATCC 12315 (DSM 20072).

2. *Lactobacillus acidophilus* (Moro 1900) Hansen and Moequot 1970, 326.<sup>AL†</sup> (*Bacillus acidophilus* Moro 1900, 115.)*acidophilus* M.L. n. *acidum* acid; Gr. adj. *philus* loving; M.L. adj. *acidophilus* acid-loving.

Rods with rounded ends, generally 0.6–0.9 × 1.5–6 µm, occurring singly, in pairs and in short chains.

With rare exceptions good growth at 45°C. Starch is fermented by most strains.

\* VP denotes that this name has been validly published in the official publication, International Journal of Systematic Bacteriology.

† AL denotes the inclusion of this name on the Approved Lists of Bacterial Names.



Table 14.4  
Differential characteristics of the obligately heterofermentative species of the genus *Lactobacillus*\*

Species	Mol% G + C	Opt in peptidoglycan	Xylose	Melibiose	Mannose	Melzitose	Arabinose	Starch capsule	Cellobiose	Mannitol	Maltose	Sucrose	NH <sub>3</sub> from arginine	Growth at 16°C	D,L-Lactic acid	L(+)-Lactic acid	Ribose	mDpm in peptidoglycan	CO <sub>2</sub> + H <sub>2</sub> from lactate
27. <i>L. bifementans</i>	46																		+
32. <i>L. divergens</i>	54																		+
49. <i>L. vacuolatus</i>	36																		+
44. <i>L. viridescens</i>	43																		+
42. <i>L. sanfrancisco</i>	37																		+
36. <i>L. fructosus</i>	47																		+
33. <i>L. fermentum</i>	53																		+
41. <i>L. reuteri</i>	41																		+
31. <i>L. confusus</i>	46																		+
40. <i>L. miror</i>	44																		+
38. <i>L. kazdleri</i>	39																		+
29. <i>L. buchneri</i>	45																		+
28. <i>L. brevis</i>	46																		+
30. <i>L. collinoides</i>	45																		+
38. <i>L. halobolus</i>	45																		+
30. <i>L. hefir</i>	41																		+
37. <i>L. hilgardii</i>	40																		+
34. <i>L. fructivorans</i>	40																		+

\* Symbols as in Table 14.3.

Table 14.5.

Pattern of fermented carbohydrates of the obligately homofermentative species of the genus *Lactobacillus* (group 1)\*

Species	Amygdalin	Arabinose	Cellobiose	Esculin	Fructose	Galactose	Glucose	Glucosate	Lactose	Maltose	Mannitol	Mannose	Melezitose	Melibiose	Raffinose	Rhamnose	Rhose	Salicin	Sorbitol	Sucrose	Trehalose	Xylose
1a. <i>L. delbrueckii</i> subsp. <i>delbrueckii</i>	+	+	p	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1b. <i>L. delbrueckii</i> subsp. <i>lactis</i>	+	+	p	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1c. <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2. <i>L. acidophilus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3. <i>L. amylophilus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4. <i>L. amylovarus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5. <i>L. animalis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6. <i>L. crispatus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7. <i>L. farciminis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8. <i>L. gasseri</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9. <i>L. helveticus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10. <i>L. jensenii</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11. <i>L. ruminis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12. <i>L. salivarius</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13. <i>L. sharpiae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14. <i>L. vitulinus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15. <i>L. yamaguchii</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\* Symbols: +, 90% or more strains positive; -, 90% of more strains negative; p, 11-99% strains positive; +, positive to weak reaction.

\* See text.

Table 14.6.

Physiological and biochemical characteristics of the obligately homofermentative species of the genus *Lactobacillus* (Group I)\*

Species	Peptidoglycan type*	Telcholic acid	Electrophoretic mobility <sup>b</sup> D-LDH L-LDH	Allosteric L-LDH	Mol% G + C	Lactic acid isomer(s) <sup>d</sup>	Growth at 15°C	NH <sub>2</sub> from arginine
1a. <i>L. delbrueckii</i> subsp. <i>delbrueckii</i>	Lys-DAsp	Glycerol	1.50	—	—	49-51 D	—	d
1b. <i>L. delbrueckii</i> subsp. <i>lactis</i>	Lys-DAsp	Glycerol	1.50	—	—	49-51 D	—	d
1c. <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	Lys-DAsp	Glycerol	1.70	—	—	49-51 D	—	—
2. <i>L. acidophilus</i>	Lys-DAsp	Glycerol	1.50 <sup>c</sup>	1.30	—	34-37 DL	—	—
3. <i>L. amylophilus</i>	Lys-DAsp	None	1.50	1.40	—	44-46 L	+	ND
4. <i>L. amylovorus</i>	Lys-DAsp	Glycerol	1.15	1.20	—	40-41 DL	—	ND
5. <i>L. animalis</i>	Lys-DAsp	None	—	1.50	—	41-44 L	—	—
6. <i>L. crispatus</i>	Lys-DAsp	Glycerol	1.35	1.10	—	35-38 DL	—	—
7. <i>L. farciminis</i>	Lys-DAsp	None	1.15	1.20	—	34-36 L(D)	+	+
8. <i>L. gasseri</i>	Lys-DAsp	None	1.35 <sup>c</sup>	0.95	—	33-35 DL	—	—
9. <i>L. helveticus</i>	Lys-DAsp	Glycerol	0.95	1.30	—	38-40 DL	—	—
10. <i>L. jensenii</i>	Lys-DAsp	Glycerol	1.50	—	—	35-37 D	—	+
11. <i>L. ruminis</i>	mDAP-Direct	None	ND	ND	—	44-47 L	—	—
12. <i>L. salivarius</i>	Lys-DAsp	None	—	1.35	—	34-36 L	—	—
13. <i>L. sharpae</i>	mDAP-Direct	Glycerol	1.34	1.48	—	53 L	+	—
14. <i>L. vitulinus</i>	mDAP-Direct	None	ND	ND	—	34-37 D	—	—
15. <i>L. yamanashiensis</i>	mDAP-Direct	None	ND	ND	—	32-34 L	+	—

\* Symbols: see Table 14.5; and ND, not determined.

<sup>b</sup> Abbreviations used by Schleifer and Kandler (1972).<sup>c</sup> Determined in polyacrylamide disk gel electrophoresis pH 7.5; L-LDH rabbit Iso I served as reference.<sup>d</sup> D or L, the isomer recorded makes up 90% or more of total lactic acid; DL, 25-75% of total lactic acid are of the L-configuration; and D(L) or L(D), the isomer given in brackets makes up 15-20% of total lactic acid.

\* Strains of this species are known to give more than one band; the migration distance recorded is that obtained with the type strain.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

Nutritional requirements: calcium pantothenate, folic acid, niacin and riboflavin are essential; pyridoxal, thiamine, thymidine and vitamin B<sub>12</sub> are not required.

DNA/DNA homology: the species comprises at least three homology groups which cannot be separated by simple phenotypical characteristics (groups A-1, A-3, A-4 of Johnson et al., 1980; groups 1a, 1b, 1d, 1e of Lauer et al., 1980). Between individual strains of the different groups DNA/DNA homology values of about 20-50% are found. Group A-1 or group 1a, respectively, which include the type strain of *L. acidophilus*, can be differentiated from the other groups by studying the electrophoretic or immunological behavior of the L-LDH. Group A-2 of Johnson et al. (1980) and the corresponding group 1c of Lauer et al. (1980) were recently found to be homologous with *L. crispatus* (Cato et al., 1983). Among the lactobacilli species of group I (thermobacteria) having a similar mol% G + C as *L. acidophilus*, only very low DNA/DNA homology of *L. acidophilus* with *L. gasseri*, *L. helveticus* and *L. crispatus* but no homology with *L. salivarius* and *L. jensenii* could be detected.

Isolated from the intestinal tract of humans and animals, human mouth and vagina.

The mol% G + C of the DNA is 32-37 (Bd, T<sub>m</sub>).

Type strain: ATCC 4356.

Further comments. *L. acidophilus* cannot be differentiated reliably from *L. gasseri*, *L. crispatus*, and *L. amylovorus* by any simple phenotypic test; electrophoretic analysis of soluble cellular proteins or lactate dehydrogenases, detailed cell wall studies or, in the case of *L. amylovorus*, determination of mol% G + C of the DNA are necessary.

3. *Lactobacillus amylophilus* Nakamura and Crowell 1981, 216.<sup>VP</sup> (Effective publication: Nakamura and Crowell 1979, 539.)

*a.myl'o'phi'lus*. Gr. n. *amylum* starch; Gr. adj. *philus* loving; M.L. adj. *amylophilus* starch loving.

Thin rods, 0.5-0.7 × 2-3 μm, occurring singly and in short chains.

No growth at 45°C. Actively ferments starch and displays extracellular amylolytic enzyme activity.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

Growth factor requirements: riboflavin, pyridoxal, pantothenic acid, niacin, and folic acid are essential; thiamine is not required.

DNA/DNA homology: four strains form a narrow homology group not related to a number of homofermentative lactobacilli species studied (Nakamura, 1982).

Isolated from swine waste-corn fermentation.

The mol% G + C of the DNA is 44-46 (Bd).

Type strain: NRRL B-4437.

4. *Lactobacillus amylovorus* Nakamura 1981, 61.<sup>VP</sup>

*a.myl'o.vo'rus*. Gr. n. *amylum* starch; *L. v. vorare* to devour; M.L. adj. *amylovorus* starch destroying.

Rods, 1 × 3-5 μm, occurring singly and in short chains. Good growth at 45°C. Actively ferments starch and displays extracellular amylolytic enzyme activity.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

Growth factor requirements: niacin, pantothenic acid, folic acid and riboflavin are essential; thiamine is not required.

DNA/DNA homology: three strains form a narrow homology group not related to the type strains of *L. acidophilus*, *L. leichmannii* and *L. amylophilus* (Nakamura, 1981).

Isolated from cattle waste-corn fermentation.

The mol% G + C of the DNA is 40.3 ± 0.1 (Bd).

Type strain: NRRL B-4540.

Further comments. Since many strains of *L. acidophilus*, *L. crispatus* and *L. gasseri* are able to ferment starch (Johnson et al., 1980), *L. amylovorus* cannot be reliably differentiated from these species by simple tests.

5. *Lactobacillus animalis* Dent and Williams 1983, 439.<sup>VP</sup> (Effective publication: Dent and Williams 1982, 384.)

*animalis*, L. n. *animalis*; L. gen. n. *animalis* of an animal. Rods with rounded ends; generally 1.0–1.2 × 3–6 µm, occurring singly or in pairs.

Good growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

Isolated from dental plaques and alimentary canal of animals.

The mol% G + C of the DNA is 41–44 (*T<sub>m</sub>*).

Type strain: NCD 2425.

Further comments. Some of the strains on which the description of *L. animalis* was based originally ferment arabinose and also ribose weakly thus resembling *L. murinus*. DNA/DNA homology studies should be directed towards establishing the genomic relationship of the different strains of *L. animalis* among each other and with *L. murinus*.

6. *Lactobacillus crispatus* (Brygoo and Aladame 1953) Moora and Holdeman 1970, 15.<sup>AL</sup> (*Eubacterium crispatum* Brygoo and Aladame 1953, 641.)

Note. An emended description of *L. crispatus* is given by Cato et al. (1983).

*crispatus*, L. part. adj. *crispatus* curled, crisped, referring to morphology observed originally in broth media.

Straight to slightly curved rods with rounded ends, 0.8–1.6 × 2.3–11 µm, occurring singly and in short chains.

Generally good growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: *L. crispatus* was found highly homologous with "*L. acidophilus*" group A-2 of Johnson et al. 1980 (Cato et al. 1983).

Isolated from human feces, vagina and buccal cavities, crops and caeca of chicken; also found in patients with purulent pleurisy, leucorrhea and urinary tract infection.

The mol% G + C of the DNA is 35–38 (*T<sub>m</sub>*).

Type strain: VPI 3199 (ATCC 33820).

Further comments. *L. crispatus* cannot reliably be differentiated from *L. acidophilus*, *L. gasseri* and *L. amylovorus* by any simple test: electrophoretic characterization of soluble cellular proteins or lactic acid dehydrogenases, detailed cell wall studies or, in the case of *L. amylovorus*, determination of mol% G + C of the DNA are necessary.

7. *Lactobacillus farciminis* Reuter 1983, 672.<sup>VP</sup> (Effective publication: Reuter 1983, 278.)

*farciminis*, L. n. *farciminis* sausage; L. gen. n. *farciminis* of sausage. Slender rods, 0.6–0.8 × 2–5 µm, occurring singly and in short chains.

No growth at 45°C. Grows in the presence of 10% NaCl and occasionally 12% NaCl.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: no genomic relationship between *L. farciminis* and other species of group II (streptobacteria; Dellaglio et al., 1975). However, 26–28% DNA/DNA homology with *L. alimentarius* has been detected by Kagameier et al. (1985).

Isolated from meat products (raw sausages) and sour dough.

The mol% G + C of the DNA is 34–36 (*T<sub>m</sub>*).

Type strain: DSM 20184 (ATCC 29644).

8. *Lactobacillus gasseri* Lauer and Kandler 1980a, 601.<sup>VP</sup> (Effective publication: Lauer and Kandler 1980, 77.)

*gasseri*, M.L. gen. n. *gasseri* of Gasser; named for F. Gasser, a French bacteriologist.

Rods with rounded ends, generally 0.6–0.8 × 3.0–5.0 µm, occurring singly and in chains. Formation of "mini cells" and snakes is frequently observed.

Generally good growth at 45°C. Starch is fermented by most strains.

Unlike all other lactobacilli, the D-alanyl-D-alanine termini of pep-

tide subunits of peptidoglycan not involved in cross-linkage are preserved because of the lack of D,D-carboxypeptidase action.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: the species comprises two DNA/DNA homology groups which cannot be separated by phenotypical characteristics (groups B-1 and B-2 of Johnson et al. 1980; groups IIa and IIb of Lauer et al. 1980). Between individual strains of the two groups, DNA/DNA homology values of about 30–60% are found. Among the species of group I (thermobacteria) having a similar mol% G + C to *L. gasseri*, only low DNA/DNA homology with *L. acidophilus* and *L. crispatus* but no homology with *L. helveticus*, *L. jensenii* and *L. salivarius* could be detected.

Isolated from the human mouth and vagina and from the intestinal tract of man and animals; also found in wounds, urine, blood and pus of patients suffering from septic infections.

The mol% G + C of the DNA is 33–35 (*T<sub>m</sub>*).

Type strain: DSM 20243.

Further comments. *L. gasseri* cannot be differentiated reliably from *L. acidophilus*, *L. crispatus* and *L. amylovorus* by any simple phenotypic test; electrophoretic analysis of soluble cellular proteins or lactate dehydrogenases, detailed cell wall studies or, in the case of *L. amylovorus*, determination of mol% G + C of the DNA are required.

9. *Lactobacillus helveticus* (Orla-Jensen 1919) Bergey, Harrison, Breed, Hammer and Hinton 1925 184.<sup>AL</sup> (*Thermobacterium helveticum* Orla-Jensen 1919, 164.)

*helveticus*, L. adj. *helveticus* Swiss.

Good growth at 45°C; maximum growth temperature 50–52°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

Growth factor requirements: Calcium pantothenate, niacin, riboflavin, pyridoxal or pyridoxamine are essential; thiamine, folic acid, vitamin B<sub>12</sub> and thymidine are not required.

DNA/DNA homology: together with strains formerly labeled "*L. jugurti*," strains of *L. helveticus* form a narrow homology group genomically unrelated to *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis* (Simonds et al., 1971; Dellaglio et al., 1973), and *L. gasseri* (Johnson et al., 1980).

A closer phylogenetic relationship between *L. helveticus* and *L. acidophilus* is indicated by 13–44% DNA/DNA homology between the two species (Johnson et al., 1980) and by the relatively high *S<sub>as</sub>* value of 0.84 compared with the values of 0.47–0.59 found between other lactobacilli species (Stackebrandt et al., 1983).

Isolated from sour milk, cheese starter cultures and cheese, particularly Emmentaler and Gruyère cheese.

The mol% G + C of the DNA is 37–40 (Bd, *T<sub>m</sub>*).

Type strain: ATCC 16009.

10. *Lactobacillus jensenii* Gasser, Mandel and Rogosa 1970, 221.<sup>AL</sup> *jen-se-ni*, M.L. gen. n. *jensenii* of Jensen; named for S. Orla-Jensen, a Danish microbiologist.

Rods with rounded ends, 0.6–0.8 × 2.0–4.0 µm, occurring singly and in short chains.

Generally good growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: strains of *L. jensenii* form a narrow homology group genetically not related to *L. acidophilus*, *L. crispatus*, *L. delbrueckii* and *L. gasseri* (Gasser and Janvier, 1980; Johnson et al., 1980).

Isolated from human vaginal discharge and blood clot.

The mol% G + C of the DNA is 35–37 (Bd).

Type strain: ATCC 25268.

Further comments. *L. jensenii* is indistinguishable from *L. delbrueckii* by simple physiological tests. The slight difference in the migration distance of D-LDH of *L. jensenii* and *L. delbrueckii* in starch gel electrophoresis observed by Gasser (1970) could not be demonstrated.

by the polyacrylamide disk gel electrophoresis routinely used in our laboratory. Therefore, determination of mol% G + C of the DNA remains the most reliable characteristic to differentiate *L. jensenii* from *L. delbrueckii*.

11. *Lactobacillus ruminis* Sharpe, Latham, Garvie, Zirngibl and Kandler 1978, 47.<sup>AL</sup>  
*ruminis*. *L. n. rumen* throat; M.L. *n. rumen* rumen; M.L. *gen n. ruminis* of rumen.

Rods, 0.6–0.8 × 3–5 µm, occurring singly, in pairs and in short chains. Motile by peritrichous flagella; motility not always easy to demonstrate and often sluggish, best demonstrated as stab cultures in semi-solid media containing low concentrations of glucose.

Surface growth is obtained only under reduced oxygen pressure; growth in liquid media is improved by the addition of cysteine-HCl.

Unlike the strains isolated from the rumen, many strains from sewage were nonmotile and failed to grow at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: all strains studied form a narrow homology group not related to others, especially *meso*-DAP-containing species (Sharpe and Dellaglio, 1977; Weiss et al., 1981).

Isolated from rumen of cow and from sewage.

The mol% G + C of the DNA is 44–47 (T<sub>m</sub>).

Type strain: ATCC 27780.

12. *Lactobacillus salivarius* Rogosa, Wiseman, Mitchell and Disraely 1953, 691.<sup>AL</sup>

*salivarius*. *L. adj. salivarius* salivary.

Rods with rounded ends, 0.6–0.9 × 1.5–5 µm, occurring singly and in chains of varying length.

Generally growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: one strain tested showed no genomic relationship to *L. acidophilus* and *L. gasseri* (Lauer et al., 1980), *L. murinus* (E. Lauer, unpublished) or *L. sake*, *L. curvatus*, and *L. furciminis* (Kagermeier et al., 1985).

Isolated from the mouth and intestinal tract of man and hamster; intestinal tract of chicken.

The mol% G + C of the DNA is 34–36 (Bd).

Two subspecies are recognized.

12a. *Lactobacillus salivarius* subsp. *salivarius* Rogosa, Wiseman, Mitchell and Disraely 1953, 691.<sup>AL</sup>

Description as for the species.

Ferments rhamnose but not salicin and esculin.

Type strain: ATCC 11741.

12b. *Lactobacillus salivarius* subsp. *salicinius* Rogosa, Wiseman, Mitchell and Disraely 1953, 691.<sup>AL</sup>

*salicinius*. M.L. *adj. salicinius* pertaining to salicin, a glycoside.

Description as for the species.

Ferments salicin and esculin but not rhamnose.

Type strain: ATCC 11742.

13. *Lactobacillus sharpae* Weiss, Schillinger, Laternser and Kandler 1982, 266.<sup>VP</sup> (Effective publication: Weiss, Schillinger, Laternser and Kandler 1981, 251.)

*sharpae*. M.L. *gen n. sharpae* of Sharpe; named for M. Elisabeth Sharpe, an English bacteriologist.

Rods with rounded ends; generally 0.6–0.8 × 2–8 µm, with a pronounced tendency to form "snakes" and, after prolonged incubation, long characteristically wrinkled chains. In broth cultures, a flocculent sediment is observed.

No growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: three strains tested proved to be completely homologous to each other, whereas one single strain was more distantly related showing only 53% homology to the type strain. No genomic relationship could be detected to other *meso*-DAP-containing species of lactobacilli (Weiss et al., 1981).

Habitat unknown, isolated from municipal sewage.

The mol% G + C of the DNA is 53 (T<sub>m</sub>).

Type strain: DSM 20505.

14. *Lactobacillus vitulinus* Sharpe, Latham, Garvie, Zirngibl and Kandler 1973, 47.<sup>AL</sup>

*vitulinus*. *L. adj. vitulinus* of a calf.

Rods with rounded ends, 0.5–0.7 × 2–4 µm, occurring singly and in pairs.

Surface growth is only obtained under anaerobic conditions; grows in freshly boiled MRS broth with (w/v) 0.05% cysteine-HCl added.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: five strains belonged to three different homology groups completely unrelated to each other. No homology was detected to possibly related species (Sharpe and Dellaglio, 1977). Since no phenotypic characteristics are presently known to separate the different homology groups, *L. vitulinus* remains genotypically heterogeneous.

Isolated from bovine rumen.

The mol% G + C of the DNA is 34–37 (T<sub>m</sub>).

Type strain: ATCC 27783.

15. *Lactobacillus yamanashiensis* Nonomura 1983, 406.<sup>VP</sup> (*Lactobacillus mali* Carr and Davies 1970, 774.)

*yamanashiensis*. M.L. *adj. yamanashiensis* belonging to Yamanashi Prefecture, Japan, the source of wine must from which the organism was isolated.

Rods, 0.6–0.8 × 2–4 µm, occurring singly, in pairs, and in short chains.

Motile with a few peritrichous flagella; motility often sluggish, best demonstrated in semisolid MRS agar stab culture with only (w/v) 0.1% glucose. Most strains exhibit a weak pseudocatalase activity when grown on MRS agar containing (w/v) 0.1% glucose; benzidine test negative.

No growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.5 and 14.6.

DNA/DNA homology: strains of *L. mali* exhibited 70–85% homology between each other and with the type strain of *L. yamanashiensis* (Carr et al., 1977). No genomic relationship was detected to strains of group II (streptobacteria; Dellaglio et al., 1975) and to other *meso*-DAP-containing L(+)-lactic acid-producing lactobacilli (Weiss et al., 1981).

Isolated from cider and wine must.

The mol% G + C of the DNA is 32–34 (T<sub>m</sub>).

Type strain: ATCC 27304.

Further comments. Nonomura (1983) mentioned two subspecies, namely *L. yamanashiensis* subsp. *yamanashiensis* and *L. yamanashiensis* subsp. *mali* in the title of the paper but, inconsequently, in the text only a description of the species, but not of the subspecies is given. The proposal of the subspecies *L. yamanashiensis* subsp. *mali* by Carr et al. (1977) is invalid since it is not mentioned in the Approved Lists of Bacterial Names (Skerman et al., 1980). ATCC 27502 is listed in the Approved Lists of Bacterial Names as type strain of *L. mali*.

Note. Significant amounts of menaquinones, predominantly with eight and nine isoprene units (MK-8, MK-9) have been found in *L. yamanashiensis* (Collins and Jones, 1981). All other lactobacilli studied so far lack both menaquinones and ubiquinones.

16. *Lactobacillus agilis* Weiss, Schillinger, Laternser and Kandler 1982, 266.<sup>VP</sup> (Effective publication: Weiss, Schillinger, Laternser and Kandler 1981, 252.)

*agilis*. *L. adj. agilis* agile; motile.

Rods with rounded ends,  $0.7-1.0 \times 3-6 \mu\text{m}$ , occurring singly, in pairs and in short chains.

Motile with peritrichous flagella; motility normally easy to demonstrate in MRS broth.

Good growth at  $45^\circ\text{C}$ .

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

DNA/DNA homology: five strains form a narrow homology group not related to representatives of any of the *meso*-DAP-containing and L(+)-lactic acid-forming species of lactobacilli (Weiss et al., 1981).

Habitat unknown, isolated from municipal sewage.

The mol% G + C is 43-44 ( $T_m$ ).

Type strain: DSM 20509.

Further comment: "*Lactobacillus plantarum* var. *mobilis*" isolated from feces of turkey (Harrison and Hansen 1950) was only tentatively named and therefore omitted from the Approved Lists of Bacterial Names (Skerman et al., 1980). According to the original description and later investigations (Sharpe et al., 1978b) this organism may belong to *L. cagilis*.

17. *Lactobacillus alimentarius* Reuter 1983, 672.<sup>VP</sup> (Effective publication: Reuter 1983, 278.)

*a.li.men.ta'ri.us*. L. adj. *alimentarius* pertaining to food.

Short, slender rods, generally  $0.6-0.8 \times 1.5-2.5 \mu\text{m}$ .

No growth at  $45^\circ\text{C}$ . Growth in the presence of 10% NaCl. Acetoin is produced from glucose.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

DNA/DNA homology: no genomic relationship was found between *L. alimentarius* and other species of group II (streptobacteria; Dellaglio et al., 1975); however, 26-28% DNA/DNA homology with *L. farciminis* was detected by Kagermeier et al. (1985).

Isolated from marinated fish products, meat products (raw sausages and sliced prepacked sausages) and sour dough.

The mol% G + C of the DNA is 36-37 ( $T_m$ ).

Type strain: DSM 20249 (ATCC 29643).

18. *Lactobacillus bavaricus* Stetter and Stetter 1980, 601.<sup>VP</sup> (Effective publication: Stetter and Stetter 1980, 73.)

*ba.va'ri.cus*. M.L. adj. *bavaricus* Bavarian.

Rods with rounded ends,  $0.8-1.0 \times \sim 2-7 \mu\text{m}$ , occurring singly and in short chains; slightly curved, especially during stationary growth phase.

No growth at  $45^\circ\text{C}$ ; growth from  $2-37^\circ\text{C}$ .

L-LDH is activated by FDP and  $\text{Mn}^{2+}$ . Does not contain lactic acid racemase.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Isolated from sauerkraut and fermented cabbage leaves.

The mol% G + C of the DNA is 41-43 ( $T_m$ ).

Type strain: ATCC 31063.

Further comments. The type strain of *L. bavaricus* as well as five additional strains tested showed 80-95% DNA/DNA homology with *L. sake*, whereas one strain was completely homologous with *L. curvatus* (Kagermeier et al., 1985). Therefore, organisms allocated to *L. bavaricus* may be regarded as racemase-free subspecies of *L. sake* or *L. curvatus*, respectively, rather than as members of a separate species. However, further studies are required before a formal description of two subspecies is possible.

19. *Lactobacillus casei* (Orla-Jensen 1916) Hansen and Lessel 1971, 71.<sup>AL</sup> (*Streptobacterium casei* Orla-Jensen 1919, 166.)

*ca'sei*. L. n. *caseus* cheese; L. gen. n. *casei* of cheese.

Rods, generally  $0.7-1.1 \times 2.0-4.0 \mu\text{m}$ , often with square ends and tending to form chains.

No growth at  $45^\circ\text{C}$  (exception: *L. casei* subsp. *rhamnosus*).

L-LDH is activated by FDP and  $\text{Mn}^{2+}$ .

Growth factor requirements: riboflavin, folic acid, calcium panto-

thoic acid and niacin are essential; pyridoxal or pyridoxamine is essential or stimulatory; thiamine, vitamin B<sub>12</sub> and thymidine are not required.

The mol% G + C of the DNA is 45-47 (Bd).

Isolated from milk and cheese, dairy products and dairy environments, sour dough, cow dung, silage, human intestinal tract, mouth and vagina, sewage.

Four subspecies are recognized within this species:

19a. *Lactobacillus casei* subsp. *casei* (Orla-Jensen 1916) Hansen and Lessel 1971, 71.<sup>AL</sup>

Description as for the species.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Type strain: ATCC 393.

Note. The lactose-negative variant labeled *Lactobacillus casei* subsp. *lactosus* Mills and Lessel 1973, 67, should no longer be regarded as a separate taxon but included in *L. casei* subsp. *casei*.

19b. *Lactobacillus casei* subsp. *pseudoplanarium* Abo-Elnaga and Kandler 1965a, 26.<sup>AL</sup>

*pseu'do.plan.ta'rium*. Gr. adj. *pseudes* false; M.L. gen. n. *planarium* a specific epithet; M.L. adj. *pseudoplanarium* not the true (*L.*) *planarium*.

Inactive lactic acid is produced due to the activity of a L-lactic acid racemase (Stetter and Kandler 1973).

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Type strain: ATCC 25598.

19c. *Lactobacillus casei* subsp. *rhamnosus* Hansen 1968, 76.<sup>AL</sup>

*rham.no'sus*. M.L. adj. *rhamnosus* pertaining to rhamnose.

These organisms are the only homofermentative lactobacilli which grow well at both  $15^\circ\text{C}$  and  $45^\circ\text{C}$ .

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Type strain: ATCC 7469.

19d. *Lactobacillus casei* subsp. *tolerans* Abo-Elnaga and Kandler 1965a, 26.<sup>AL</sup>

*to.le.rans*. L. pres. part. *tolerans* tolerating, enduring; means survival during pasteurization of milk.

Survives heating at  $72^\circ\text{C}$  for 40 s and ferments a very narrow range of carbohydrates.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Type strain: ATCC 25599.

DNA/DNA homology: except the type strain of *L. casei* subsp. *casei* and the members of *L. casei* subsp. *rhamnosus*, all *L. casei* form a narrow homology group genomically not related to other species of group II (streptobacteria; Dellaglio et al., 1975). The type strain of *L. casei* subsp. *casei* is highly homologous only with "*Lactobacterium zeae*" whereas homology with other strains of *L. casei* is significantly lower than 50% indicating a heterogeneity of the species.

The strains of *L. casei* subsp. *rhamnosus* highly homologous among each other display only 30-50% homology with strains of other subspecies of *L. casei*. Thus, *L. casei* subsp. *rhamnosus* deserves the rank of a separate species rather than that of a subspecies of *L. casei*.

20. *Lactobacillus coryniformis* Abo-Elnaga and Kandler 1965a, 18.<sup>AL</sup>

*co.ry'ni.for'mis*. Gr. n. *coryne* a club; L. adj. *formis* shaped; M.L. adj. *coryniformis* club-shaped.

Short, often coccoid, rods; frequently somewhat pear-shaped,  $0.8-1.1 \times 1-3 \mu\text{m}$ , occurring singly, in pairs or short chains.

Generally no growth at  $45^\circ\text{C}$ .

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Growth factor requirements: pantothenic acid, niacin, riboflavin,



Table 14.7.  
Pattern of fermented carbohydrates of the facultatively heterofermentative species of the genus *Laetobacillus* (group II)\*

Species	Amygdalin	Arabinose	Cellobiose	Esculin	Fructose	Galactose	Glucose	Gluconate	Lactose	Maltose	Mannitol	Mannose	Melzitose	Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Sucrose	Trehalose	Xylose
16. <i>L. agilis</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17. <i>L. alimentarius</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18. <i>L. bavaricus</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19a. <i>L. casei</i> subsp. <i>casei</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19b. <i>L. casei</i> subsp. <i>pseudoplanitarius</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19c. <i>L. casei</i> subsp. <i>rhinno-</i> <i>side</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19d. <i>L. casei</i> subsp. <i>tolerans</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20a. <i>L. coryniformis</i> subsp. <i>coryniformis</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20b. <i>L. coryniformis</i> subsp. <i>torquens</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21. <i>L. curvatus</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22. <i>L. homotrichii</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23. <i>L. maltaromicus</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24. <i>L. murinus</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25. <i>L. plantarium</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26. <i>L. solis</i>	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\* Symbols: see Table 14.5; and of reaction not determined.

\* See text.

Table 14.8.

Physiological and biochemical characteristics of the facultatively heterofermentative species of the genus *Lactobacillus* (group II)\*

Species	Peptidoglycan type <sup>b</sup>	Teichoic acid	Electrophoretic mobility <sup>c</sup>		Allosteric L-LDH	Mol% G + C	Lactic acid isomer(s) <sup>d</sup>	Growth at 15°C	NH <sub>3</sub> from arginine
			D-LDH	L-LDH					
16. <i>L. agilis</i>	mDAP-Direct	None	1.40	1.20	—	42-44	L	—	—
17. <i>L. alimentarius</i>	Lys-DAsp	None	0.80	1.10	—	36-37	L(D)	+	—
18. <i>L. bavaricus</i>	Lys-DAsp	None	—	1.60	+	41-43	L	+	—
19a. <i>L. casei</i> subsp. <i>casei</i>	Lys-DAsp	None	1.22 <sup>e</sup>	0.93	+	45-47	L	+	—
19b. <i>L. casei</i> subsp. <i>pseudoplantarum</i>	Lys-DAsp	None	1.04	0.93	+	45-47	DL	+	—
19c. <i>L. casei</i> subsp. <i>rhamnosus</i>	Lys-DAsp	None	0.75	0.93	+	45-47	L	+	—
19d. <i>L. casei</i> subsp. <i>tolerans</i>	Lys-DAsp	None	—	0.93	+	45-47	L	+	—
20a. <i>L. coryniformis</i> subsp. <i>coryniformis</i>	Lys-DAsp	None	0.38	—	—	45	D(L)	+	—
20b. <i>L. coryniformis</i> subsp. <i>torquens</i>	Lys-DAsp	None	0.38	—	—	45	D	+	—
21. <i>L. curvatus</i>	Lys-DAsp	None	1.20	1.60	+	42-44	DL	+	—
22. <i>L. homohiochii</i>	Lys-DAsp	Glycerol	ND	ND	—	35-38	DL	+	—
23. <i>L. maliaromicus</i>	mDAP-Direct	None	ND	ND	—	36	L	+	ND
24. <i>L. murinus</i>	Lys-DAsp	None	—	0.92	+	43-44	L	—	—
25. <i>L. plantarum</i>	mDAP-Direct	Ribitol or glycerol	1.44	1.28	—	44-46	DL	+	—
25. <i>L. sake</i>	Lys-DAsp	None	1.20	1.60	+	42-44	DL	+	—

\* Symbols: see Table 14.5; and ND, not determined.

<sup>b</sup> Footnotes: see Table 14.6.

biotin and *p*-aminobenzoic acid are essential for all or the majority of the strains tested; folic acid, pyridoxin, thiamine and vitamin B<sub>12</sub> are not required.

DNA/DNA homology: four strains representing both subspecies are highly homologous among each other, but no genomic relationship to other species of group II is found (Dellaglio et al., 1975).

Isolated from silage, cow dung, dairy barn air and sewage.

The mol% G + C of the DNA is close to 45 (*T<sub>m</sub>*).

Two subspecies are recognized within *L. coryniformis*.

20a. *Lactobacillus coryniformis* subsp. *coryniformis* Abo-Elnaga and Kandler 1965a, 18.<sup>4c</sup>

The lactic acid produced from glucose contains appreciable amounts of the L-isomer (15-20% of total lactic acid).

Type strain: DSM 20001.

20b. *Lactobacillus coryniformis* subsp. *torquens* Abo-Elnaga and Kandler 1965a, 18.<sup>4c</sup>

*torquens*, *L.* pres. part. *torquens* twisting.

Exclusively D(-)-lactic acid is produced.

Type strain: ATCC 25600.

21. *Lactobacillus curvatus* (Troili-Petersson 1903) Abo-Elnaga and Kandler 1965a, 19.<sup>4c</sup> (*Bacterium curvatum* Troili-Petersson 1903, 137.)

*curva'tus*, *L. v. curvare* to curve; *L.* pres. part. *curvatus* curved.

Curved, bean-shaped rods with rounded ends, 0.7-0.9 × 1-2 μm, occurring in pairs and short chains; closed rings of usually four cells or horseshoe forms frequently observed. Some strains at first motile; motility lost on subculture.

No growth at 45°C; some strains tested are able to grow even at 2-4°C.

L-LDH is activated by FDP and Mn<sup>2+</sup>. Possesses lactic acid racemase whose biosynthesis is induced by L(+)-lactic acid. Racemase induction generally not repressed by acetate.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

DNA/DNA homology: strains of *L. curvatus* form a narrow homology group not related to other lactobacilli species except *L. sake*; *L. curvatus*

and *L. sake* have 40-50% homology with each other (Dellaglio et al., 1975; Kagermeier et al., 1985).

Isolated from cowdung, milk, silage, sauerkraut, prepacked finished dough and meat products.

The mol% G + C of the DNA is 42-44 (*T<sub>m</sub>*).

Type strain: ATCC 25601.

Note. Some of the atypical streptobacteria from herbage, silage, fermented meat products and vacuum-packaged meat reported in the past belong to *L. curvatus*.

22. *Lactobacillus homohiochii* Kitahara, Kaneko and Goto 1957, 116.<sup>4c</sup>

*ho'mo.hi.o'chi.i*, Gr. adj. *homos* like, equal; Japanese *n. hiochi* spoiled sake; M.L. gen. *n. homohiochii* probably intended to mean homofermentative lactobacillus of hiochi.

Rods, with rounded ends, 0.7-0.8 × 2-4 μm or, occasionally, 6 μm in length.

Does not grow in MRS broth. In Rogosa SL broth supplemented with DL-mevalonic acid (30 mg/liter) and ethanol (40 ml/liter) copious growth is obtained at 30°C after a marked lag phase of 4-7 days.

No growth at 45°C and at an initial pH higher than 5.5. Resistant to 13-16% ethanol.

A redetermination of the lactic acid configuration produced by the type strain in our laboratory yielded equal amounts of D- and L(+)-lactic acid.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Growth factor requirements: D-mevalonic acid is essential or highly stimulatory; ethanol is promotive.

DNA/DNA homology: the type strain of *L. homohiochii* was found to be genetically highly related to the type strain of *L. sake* but unrelated to other streptobacteria (Dellaglio et al., 1975). In our laboratory, however, two strains of *L. homohiochii* were completely homologous with each other and had only 10% homology with *L. sake* (E. Lauer, unpublished results).

Isolated from spoiled sake.

The mol% G + C of the DNA is 35-38 (*T<sub>m</sub>*). The values of 46% obtained by chemical analysis (Gasser and Sebald, 1966) and by *T<sub>m</sub>*

(eighth edition Bergey's *Manual*) are in contrast with the values found by Momose et al., 1974 (34.6–36.8%) and our own unpublished results (38%).

Type strain: ATCC 15434.

23. *Lactobacillus maltaromicus* Miller, Morgan and Libbey 1974, 352.<sup>AL</sup>

*malt.a.ro'micus*. M.E. n. *malle* ground dried sprouted barley; *L. n.* aroma pleasant flavor; M.L. adj. *maltaromicus*, producing a maltlike aroma.

Slender rods of varying length with a tendency to form filaments and long chains.

No growth at 45°C. Besides moderate amounts of L(+) lactic acid (~1.5 g/liter) different aldehydes and alcohols such as 2-methylpropanaldehyde, 2-methylpropanol, 3-methylbutyraldehyde and 3-methylbutanol are produced in skim milk and trypticase soy broth.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Growth factor requirements: riboflavin and folic acid are essential, thiamine is not required.

DNA/DNA homology: no genetic relationship could be detected between *L. maltaromicus* and other meso-DAP-containing lactobacilli producing L(+) lactic acid (Weiss et al., 1981).

Isolated from producers' milk samples possessing a malty flavor.

The mol% G + C content of the DNA is about 36.0 (*T<sub>m</sub>*).

Type strain: ATCC 27855.

24. *Lactobacillus murinus* Hemme, Raibaud, Ducluzeau, Galpin, Sicard and van Heijenoort 1982, 384.<sup>VP</sup> (Effective publication: Hemme, Raibaud, Ducluzeau, Galpin, Sicard and van Heijenoort 1980, 306.)  
*mu.ri'nus*. *L. adj. murinus* of mice.

Rods with rounded ends, 0.8–1.0 × 2.0–4.0 μm, frequently in chains.

Good growth at 45°C. Ribose and arabinose slowly fermented. L-LDH is activated by FDP and Mn<sup>2+</sup>.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Growth factor requirements: riboflavin is essential, thiamine and vitamin B<sub>12</sub> not required.

DNA/DNA homology: two strains tested were completely homologous to each other but unrelated to *L. alimentarius*, *L. casei*, *L. sake*, *L. curvatus* and *L. salivarius* (E. Lauer, unpublished results).

Isolated from the intestinal tract of mice and rats.

The mol% G + C of the DNA is 43.4–44.3 (*T<sub>m</sub>*).

Type strain: CNRZ 220.

25. *Lactobacillus plantarum* (Orla-Jensen 1919) Bergey, Harrison, Breed, Hammer and Hulton 1923, 250.<sup>AL</sup> (*Streptobacterium plantarum* Orla-Jensen 1919, 174.)

*plan.ta'rum*. *L. fem. n. planta* a sprout; M.L. n. *planta* a plant; M.L. gen. pl. n. *plantarum* of plants.

Rods with rounded ends, straight, generally 0.9–1.2 μm wide × 3–8 μm long, occurring singly, in pairs or in short chains.

No growth at 45°C. Some strains are able to reduce nitrate provided the concentration of glucose in the medium is limited and the pH thus poised at 6.0 or higher. Occasional strains exhibit pseudocatalase activity especially if grown under glucose limitation. Cell walls contain either ribitol or glycerol teichoic acid.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

Growth factor requirements: calcium pantothenate and niacin required; thiamine, pyridoxal or pyridoxamine, folic acid, vitamin B<sub>12</sub>, thymidine or deoxyribosides not required; riboflavin generally not required.

DNA/DNA homology: *L. plantarum* strains form two homology groups genomically related to each other at the level of 50–60%, but not related to other streptobacteria and other meso-DAP-containing lactobacilli species (Dellaglio et al., 1976; Weiss et al., 1981). "*L. pentosus*" Fred et al., 1921, and several other strains designated *L.*

*plantarum* form a third genotype only related at the 50% level to the two other genotypes. Therefore it should be regarded as a separate species (see Comments).

Isolated from dairy products and environments, silage, sauerkraut, pickled vegetables, sour dough, cow dung, and the human mouth, intestinal tract and stools, and from sewage.

The mol% G + C of the DNA is 44–46 (*B<sub>d</sub>*, *T<sub>m</sub>*).

Type strain: ATCC 14917.

Further comments. In the course of the last few years, a number of strains, mainly from sewage, have been isolated in the authors' laboratory. These strains shared high DNA/DNA homology with "*L. pentosus*" but only low homology with *L. plantarum*. Characteristically, these strains fermented glycerol whereas none of the strains within the *L. plantarum* homology group did so. A description of these organisms as *L. pentosus* nom. rev. is in preparation.

26. *Lactobacillus sake* Katagiri, Kitahara and Fukami 1934, 157.<sup>AL</sup>

*sa'ke*. Japanese n. *sake* rice wine; M.L. n. *sake* rice wine.

Rods with rounded ends, generally 0.6–0.8 × 2–3 μm, occurring singly and in short chains; frequently slightly curved and irregular, especially during stationary growth phase.

No growth at 45°C; many of the strains tested grow even at 2–4°C.

L-LDH is activated by FDP and Mn<sup>2+</sup>. Possesses lactic acid racemase; induction of racemase in most strains is repressed by acetate. Therefore, the majority of strains produce L(+) lactic acid in MRS broth whereas DL-lactic acid is produced in cabbage press juice. A few strains, whose identity with *L. sake* is confirmed by DNA/DNA homology, however, produce inactive lactic acid, also, in MRS broth.

Additional physiological and biochemical characteristics are presented in Tables 14.7 and 14.8.

DNA/DNA homology: the type strain and many other strains form a narrow homology group not significantly related to other lactobacilli except *L. bavaricus* (Kagermeier et al., 1985) and *L. curvatus* (Dellaglio et al., 1975; Kagermeier et al., 1985). While most strains of *L. bavaricus* exhibit complete DNA/DNA homology with *L. sake*, *L. curvatus* and *L. sake* are related to each other at a level of 40–50% homology. The high level of homology between *L. sake* and *L. homohiochii* reported by Dellaglio et al., (1975) could not be confirmed in our laboratory. Two strains of *L. homohiochii* completely homologous to each other show only about 10% homology to *L. sake*.

Originally isolated from sake starter; regularly found in sauerkraut and other fermented plant material, meat products and prepacked finished dough.

The mol% G + C of the DNA is 42–44 (*T<sub>m</sub>*).

Type strain: ATCC 15521.

Note. Some of the atypical streptobacteria from herbage, silage, fermented meat products and vacuum packaged meat reported in the past probably belong to *L. sake*.

27. *Lactobacillus bifermmentans* Kandler, Schillinger and Weiss 1983, 896.<sup>VP</sup> (Effective publication: Kandler, Schillinger and Weiss 1983, 409.)

*bi.fermen'tans*. *L. pref. bis* twice; *L. part. fermentans* leavening; M.L. part. adj. *bifermmentans* doubly fermenting.

Irregular rods with rounded or often tapered ends, 0.5–1.0 × 1.5–2.0 μm, occurring singly, in pairs or irregular short chains, often forming clumps.

No growth at 45°C.

Homofermentative with production of DL-lactic acid in media containing more than 1% fermentable hexoses. Lactic acid is fermented to acetic acid, ethanol, CO<sub>2</sub> and H<sub>2</sub> at pH < 4.0.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: no genomic relationship is detected between the type strain of *L. bifermmentans* and heterofermentative lactobacilli (Vescovo et al., 1979).

In contrast to all other lactobacilli *L. bifermmentans* ferments lactate

Table 14.9:

Pattern of fermented carbohydrates of the obligately heterofermentative species of the genus *Lactobacillus* (group III)\*

Species	Amygdalin	Arabinose	Cellobiose	Raculin	Fructose	Galactose	Glucose	Glucosate	Lactose	Maltose	Mannitol	Mannose	Melzitose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Sucrose	Trehalose	Xylose	
27. <i>L. bifarmicans</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28. <i>L. brevis</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29. <i>L. buchneri</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30. <i>L. collinoides</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31. <i>L. confusus</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32. <i>L. disparis</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33. <i>L. fermentum</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34. <i>L. fructivorans</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35. <i>L. fructosus</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
36. <i>L. halotolerans</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37. <i>L. hilgardii</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
38. <i>L. kimihari</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39. <i>L. lafir</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40. <i>L. minor</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
41. <i>L. rutarif</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42. <i>L. sanqranisco</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
43. <i>L. vaccinostercus</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
44. <i>L. viridescens</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\* Symbols see Tables 14.5f and o, reaction not determined.

Table 14.10.

Physiological and biochemical characteristics of obligately heterofermentative species of the genus *Lactobacillus* (group III)\*

Species	Peptidoglycan type <sup>a</sup>	Teichoic acid	Electrophoretic mobility <sup>a</sup>		Allosteric L-LDH	Mol% G + C	Lactic acid isomer(s) <sup>a</sup>	Growth at 15°C	NH <sub>2</sub> from arginine
			D-LDH	L-LDH					
27. <i>L. bifermens</i>	Lys-DAsp	None	1.10	1.20	—	45	DL	+	—
28. <i>L. brevis</i>	Lys-DAsp	Glycerol	1.62	1.40	—	44-47	DL	+	+
29. <i>L. buchneri</i>	Lys-DAsp	Glycerol	1.33	1.28	—	44-46	DL	+	+
30. <i>L. collinoides</i>	Lys-DAsp	Glycerol	1.50	1.22	—	46	DL	+	+
31. <i>L. confusus</i>	Lys-Ala	None	2.08	1.82	—	45-47	DL	+	+
32. <i>L. divergens</i>	mDAP-Direct	None	—	1.30	—	33-35	L	+	+
33. <i>L. fermentum</i>	Orn-DAsp	None	1.85	—	—	52-54	DL	—	+
34. <i>L. fructivorans</i>	Lys-DAsp	None	ND	ND	—	38-41	DL	+	+
35. <i>L. fructosus</i>	Lys-Ala	None	1.32	1.14	—	47	D(L)	+	—
36. <i>L. halotolerans</i>	Lys-Ala-Ser	Glycerol	1.75	1.80	—	45	DL	+	+
37. <i>L. hilgardii</i>	Lys-DAsp	Glycerol	1.31	0.97	—	39-41	DL	+	+
38. <i>L. kandleri</i>	Lys-Ala-Gly-Ala <sub>2</sub>	None	2.10	—	—	89	DL	+	+
39. <i>L. kefir</i>	Lys-DAsp	Glycerol	1.23	1.07	—	41-42	DL	+	+
40. <i>L. minor</i>	Lys-Ser-Ala <sub>2</sub>	Glycerol	2.08	1.50	—	44	DL	+	+
41. <i>L. reuteri</i>	Lys-DAsp	None	1.74	0.88	—	40-42	DL	—	+
42. <i>L. sanfrancisco</i>	Lys-Ala	None	1.18	1.05	—	38-38	DL	+	—
43. <i>L. vaccinostercus</i>	mDAP-Direct	ND	1.32	1.18	—	36	DL	—	—
44. <i>L. viridescens</i>	Lys-Ala-Ser	Ribitol	2.03	—	—	41-44	DL	+	—

\* Symbols: see Table 14.5; and ND, not determined.

<sup>a</sup> Footnotes: see Table 14.6.

and produces free H<sub>2</sub> and was therefore put on the list of species incertae sedis in the eighth edition of the *Manual*. 16S rRNA cataloging (Stackebrandt et al., 1983) and more recently DNA/rRNA hybridization studies (U. Schillinger, personal communication), however, gave strong evidence that *L. bifermens* belongs to the genus *Lactobacillus* (see also under Taxonomic Comments).

Isolated from spoiled Edam and Gouda cheeses where it forms undesired small cracks ("Boekelscheuren" Petts and Beynum 1948).

The mol% G + C of the DNA is 45 (T<sub>m</sub>).

Type strain: DSM 20303.

28. *Lactobacillus brevis* (Orla-Jensen 1919) Bergey, Breed, Hammer, Hinton, Murray and Harrison 1934, 312.<sup>42</sup> (*Betabacterium breve* Orla-Jensen 1919, 175.)

*brevis* L. adj. *brevis* short.

Rods with rounded ends, generally 0.7–1.0 × 2–4 µm, occurring singly and in short chains.

No growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

Growth factor requirements: Calcium pantothenate, niacin, thiamine and folic acid are essential; riboflavin, pyridoxal and vitamin B<sub>12</sub> not required.

DNA/DNA homology: only 11 out of 24 strains originally labeled *L. brevis* form a narrow homology group including the type strain of *L. brevis*. The remaining strains were found homologous with *L. hilgardii*, *L. kefir*, *L. confusus* or *L. collinoides* or remained unassigned (Vescovo et al., 1979).

Isolated from milk, cheese, sauerkraut, sour dough, silage, cow manure, feces, mouth and intestinal tract of humans and rats.

The mol% G + C of the DNA is 44–47 (Bd, T<sub>m</sub>).

Type strain: ATCC 14869.

Further comments. *L. brevis* is often difficult to distinguish clearly from *L. buchneri*, *L. hilgardii*, *L. collinoides* or *L. kefir* by simple physiological tests, especially carbohydrate fermentation reactions. In addition to DNA/DNA homology, characterization of the electrophoretic mobility of lactic acid dehydrogenases seems the most reliable procedure to separate these species.

29. *Lactobacillus buchneri* (Henneberg 1903) Bergey, Harrison,

Breed, Hammer and Hinton 1923, 251.<sup>42</sup> (*Bacillus Buchneri* (sic) Henneberg 1903, 163.)

*buchneri* M.L. gen. n. *buchneri* of Buchner; named for E. Buchner, a German bacteriologist.

Rods with rounded ends, generally 0.7–1.0 × 2–4 µm, occurring singly and in short chains.

No growth at 45°C.

*L. buchneri* is identical in almost all characteristics with *L. brevis*, except *L. buchneri* ferments melizitose and its L-LDH and D-LDH migrate distinctly slower in electrophoresis. However, at least one strain studied in detail in our laboratory did not ferment melizitose, although its LDH was electrophoretically identical with *L. buchneri*. Another strain was melizitose-positive but behaved like *L. brevis* in electrophoresis.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: in spite of the high phenotypic similarities mentioned above, six strains of *L. buchneri* formed a narrow homology group completely unrelated to *L. brevis* and other heterofermentative lactobacilli (Vescovo et al., 1979).

Isolated from milk, cheese, fermenting plant material and human mouth.

The mol% G + C of the DNA is 44–46 (Bd, T<sub>m</sub>).

Type strain: ATCC 4005.

30. *Lactobacillus collinoides* Carr and Davies 1972, 470.<sup>42</sup> *collinoides* L. adj. *collinus* hilly; Gr. n. *idus* form, shape; M.L. adj. *collinoides* hill-shaped, pertaining to colony form.

Rods with rounded ends, generally 0.6–0.8 × 3–5 µm; tendency to form long filaments, occurring singly, in palisades and irregular clumps.

No growth at 45°C. Growth in MRS broth is distinctly improved by the addition of 20% tomato juice and by replacement of glucose by maltose.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: six strains tested form a narrow homology group not related to other heterofermentative lactobacilli (Vescovo et al., 1979).

Isolated from cider.

The mol% G + C of the DNA is 46 ( $T_m$ ).

Type strain: ATCC 27612.

31. *Lactobacillus confusus* (Holzapfel and Kandler 1969) Shaibe, Garvie and Tibbory 1972, 896.<sup>AL</sup> (*Lactobacillus coprophilus* subsp. *confusus* Holzapfel and Kandler 1969, 665.)

*confusus*. L. v. *confundere* to confuse; L. past part. *confusus* confused, an allusion to its original confusion with *Leuconostoc*.

Short rods, 0.8–1.0 × 1.5–3 µm, with tendency to thicken at one end; occurring singly, rarely in short chains.

Growth at 45°C variable. Dextran is produced from sucrose.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: four strains form a narrow homology group to which two strains are only distantly related (Vescovo et al., 1979). One of the deviating strains (DSM 20194) displayed 73% homology with the type strain when reinvestigated in our laboratory. No significant genomic relationship to other heterofermentative lactobacilli was detected.

Isolated from sugarcane and carrot juice, occasionally found in raw milk, saliva and sewage.

The mol% G + C of the DNA is 45–47 (Bd,  $T_m$ ).

Type strain: ATCC 10881.

32. *Lactobacillus divergens* Holzapfel and Gerber 1984, 270.<sup>VP</sup> (Effective publication: Holzapfel and Gerber 1983, 530.)

*divergens*. L. part. *divergens* deviating, diverging.

Rods with rounded ends, 0.5–0.7 × 1.0–1.5 µm, occurring singly, in pairs and in short chains.

No growth at 45°C. Growth in MRS broth is relatively poor and visible gas is not, or only faintly, produced because of lack of an hitherto undetermined growth factor. This growth factor is contained in peptone from soybeans and certain yeast paste preparations ("Cenobis") and is produced by some molds which occur as laboratory infections. Pseudocatalase is produced on haem-containing media.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

Isolated from vacuum-packaged, refrigerated meat.

The mol% G + C of the DNA is 33–35 ( $T_m$ ).

Type strain: DSM 20623 (strain 66).

33. *Lactobacillus fermentum* Beijerinck 1901, 233.<sup>AL</sup> (*Lactobacillus cellobiosus* Rogosa, Wiseman, Mitchell and Disraeli 1953, 693.<sup>AL</sup>)

Note. Because of high phenotypic similarities and complete DNA/DNA homology, *L. cellobiosus* is here regarded as a biotype of *L. fermentum*.

*fermentum*. L. n. *fermentum* ferment, yeast.

Rods; 0.5–0.9 µm thick and highly variable in length, mostly occurring singly or in pairs.

Generally good growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

Growth factor requirements: calcium pantothenate, niacin and thiamine are essential; riboflavin, pyridoxal and folic acid not required.

DNA/DNA homology: strains of *L. fermentum* and *L. cellobiosus* form a narrow homology group not related to other heterofermentative lactobacilli (Vescovo et al., 1979).

Isolated from yeast, milk products, sour dough, fermenting plant material, manure, sewage and mouth and feces of man.

The mol% G + C of the DNA is 52–54 (Bd,  $T_m$ ).

Type strain: ATCC 14931.

Further comments. *L. fermentum* cannot be definitely distinguished from *L. reuteri* by simple physiological tests. Determinations of mol% G + C of the DNA, diaminic acid of murein and electrophoretic mobility of LDH clearly separate the two species.

34. *Lactobacillus fructivorans* Charlton, Nelson and Werkman 1934, 1.<sup>AL</sup> (*Lactobacillus trichodes* Fornachon, Douglas and Vaughn

1949, 129.<sup>AL</sup>; *Lactobacillus heterohiochii* Kitahara, Kaneko and Goto 1957, 117.<sup>AL</sup>)

Note. Because of high phenotypic and genomic similarities found between *L. fructivorans*, *L. trichodes* and *L. heterohiochii*, *L. trichodes* and *L. heterohiochii* are here regarded as junior subjective synonyms of *L. fructivorans* (see Weiss et al., 1983a).

*fructivorans*. L. n. *fructus* fruit; L. v. *vorare* to eat; M.L. pres. part. *fructivorans* fruit-eating, intended to mean fructose-devouring.

Rods with rounded ends, generally 0.5–0.8 × 1.5–4 µm, occurring singly, in pairs and in chains; very long; more or less curved or coiled filaments often observed.

No growth at 45°C.

Acidophilic; favorable pH is 5.0–5.5; no growth at an initial pH higher than 6.0.

Nutritionally very exacting, at least on primary isolation. Depending on the source of isolation, mevalonic acid, tomato juice and/or ethanol are required for growth. Some strains, especially those isolated from nonalcohol-containing sources, often become less fastidious during laboratory transfers and grow well in MRS broth.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: the type strains of *L. fructivorans*, *L. trichodes* and *L. heterohiochii* and two additional strains are highly homologous among each other and genomically not related to other heterofermentative lactobacilli (Vescovo et al., 1979; Weiss et al., 1983a). The high homology values between *L. heterohiochii* and *L. buchneri* reported by Vescovo and co-workers could not be confirmed in our laboratory and may be caused by the use of an impure or mislabeled culture of *L. heterohiochii*.

Isolated from spoiled mayonnaise, salad dressings and vinegar preserves; from spoiled sake, dessert wines and aperitifs.

The mol% G + C of the DNA is 38–40 ( $T_m$ ).

Type strain: ATCC 8288.

35. *Lactobacillus fructosus* Kodama 1956, 705.<sup>AL</sup> *fructosus*. M.L. adj. *fructosus* of fructose, pertaining to fructose.

Rods, 0.5–0.8 × 2–4 µm, occurring singly, in pairs and in short chains.

No growth at 45°C. Growth in MRS broth is markedly improved if glucose is replaced by fructose.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

Isolated from flowers.

The mol% G + C of the DNA is 47 ( $T_m$ ).

Type strain: ATCC 13162.

36. *Lactobacillus halotolerans* Kandler, Schillinger and Weiss 1983, 672.<sup>VP</sup> (Effective publication: Kandler et al., 1983, 283.)

*halotolerans*. Gr. n. *hals* salt; L. pres. part. *tolerans* tolerating, enduring; M.L. part. adj. *halotolerans* salt tolerating.

Irregular, short or even coccoid rods with rounded to tapered ends, generally 0.5–0.7 × 1–3 µm, sometimes longer, with tendency to form coiling chains, clumping together.

No growth at 45°C. Good growth in the presence of 12% NaCl and very weak growth in the presence of 14% NaCl.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: no significant genomic relationship between *L. halotolerans* and other heterofermentative lactobacilli is detected (Vescovo et al., 1979).

Isolated from meat products.

The mol% G + C of the DNA is 45 ( $T_m$ ).

Type strain: DSM 20190 (= strain R61).

37. *Lactobacillus hilgardii* Douglas and Cruess 1936, 115.<sup>AL</sup> *hilgardii*. M.L. gen. n. *hilgardii* named for Hilgard.

Rods with rounded ends, generally 0.5–0.8 × 2–4 µm, occurring singly, in short chains and frequently in long filaments.

No growth at 45°C. Optimal initial pH for growth and carbohydrate



fermentation reactions is in the range of 4.5–5.5. Grows in the presence of 15–18% ethanol.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: genomically 13 strains, mostly isolated from wine and originally allocated to a variety of different species such as *L. brevis*, "*L. desidiosus*," *L. reuteri* and "*Betabacterium vermiforme*," are highly related to the type strain of *L. hilgardii*. No relationship to other heterofermentative lactobacilli is detected (Vescovo et al., 1979).

Originally isolated from California table wines but obviously widely distributed in wines of different origin.

The mol% G + C of the DNA is 39–41 ( $T_m$ ).

Type strain: ATCC 8290.

38. *Lactobacillus kandleri* Holzapfel and van Wyk 1983, 439.<sup>VP</sup> (Effective publication: Holzapfel and van Wyk 1982, 501.)

kand'le.ri. M.L. gen. n. *kandleri* of Kandler, named for O. Kandler, a German biologist.

Partly irregular rods, generally  $0.7\text{--}0.8 \times 1\text{--}5\text{ }\mu\text{m}$ , occurring singly or in pairs, seldom in short chains.

No growth at 45°C. Slime is produced from sucrose.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

Isolated from a desert spring.

The mol% G + C of the DNA is 39 ( $T_m$ ).

Type strain: DSM 20593.

39. *Lactobacillus kefir* Kandler and Kunath 1983, 672.<sup>VP</sup> (Effective publication: Kandler and Kunath 1983, 292.)

ke'fir. Turkish n. *kefir*, a Caucasian sour milk.

Rods with rounded ends, generally  $0.6\text{--}0.8 \times 3.0\text{--}15\text{ }\mu\text{m}$ , with tendency to form chains of short rods or long filaments.

No growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: strains isolated from kefir, together with two isolates from beer, form a narrow homology group genomically unrelated to other heterofermentative lactobacilli (Vescovo et al., 1979). *L. kefir* exhibits a DNA/DNA homology of about 40% to *L. buchneri* (Kandler and Kunath, 1983).

Isolated from kefir grains and drink kefir.

The mol% G + C of the DNA is 41–42 ( $T_m$ ).

Type strain: DSM 20587 (strain A/K).

40. *Lactobacillus minor* Kandler, Schillinger and Weiss 1983, 672.<sup>VP</sup> (Effective publication: Kandler, Schillinger and Weiss 1983, 284.) (*Lactobacillus corynoides* subsp. *minor* Abo-Elnaga and Kandler 1965b, 128; *Lactobacillus viridescens* subsp. *minor* Kandler and Abo-Elnaga 1966, 754.)

mi'nor. L. comp. adj. *minor* smaller.

Irregular, short rods with rounded to tapered ends, generally  $0.6\text{--}0.8 \times 1.5\text{--}2.0\text{ }\mu\text{m}$ , sometimes longer, often bent with unilateral swellings, occurring in pairs or short chains with a tendency to form loose clusters.

No growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: no genetic relationship detected between *L. minor* and other heterofermentative lactobacilli (Vescovo et al., 1979).

Isolated from the sludge of milking machines.

The mol% G + C of the DNA is 44 ( $T_m$ ).

Type strain: DSM 20014 (strain 3).

41. *Lactobacillus reuteri* Kandler, Stetter and Köhl 1982, 266.<sup>VP</sup> (Effective publication: Kandler, Stetter and Köhl 1980, 267.) (*Lactobacillus fermentum* Type II Lérche and Reuter 1962, 462.)

reu'te.ri. M.L. gen. n. *reuteri* of Reuter, named for G. Reuter, a German bacteriologist.

Slightly irregular, bent rods with rounded ends, generally  $0.7\text{--}1.0 \times$

$2.0\text{--}5.0\text{ }\mu\text{m}$ , occurring singly, in pairs and in small clusters. Generally good growth at 45°C. In the original description, it was mistakenly stated that ammonia is not produced from arginine.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: five strains tested form a narrow homology group not related to other heterofermentative lactobacilli (Vescovo et al., 1979; Dellaglio, personal communication). In addition, 218 strains isolated from feces of milking calves and indistinguishable from *L. fermentum* by physiological tests, displayed almost complete homology with *L. reuteri* but are genetically unrelated to *L. fermentum* (Sarra et al., 1979).

Isolated from feces of humans and animals and from meat products. The mol% G + C of the DNA is 40–42.3 ( $T_m$ ).

Type strain: DSM 20018.

Note. *L. reuteri* cannot be definitely distinguished from *L. fermentum* by simple physiological tests. Determination of mol% G + C, diamino acid of peptidoglycan or electrophoretic mobility of LDH clearly separates the two species.

42. *Lactobacillus sanfrancisco* Weiss and Schillinger 1984, 503.<sup>VP</sup> (Effective publication: Weiss and Schillinger 1984, 231.)

Note. Kline and Sugihara (1971) proposed the name *L. sanfrancisco* with reservation as to results of pending DNA/DNA homology studies. Later on they confirmed briefly the proposal and designated a type strain (Sugihara and Kline, 1975). The name, however, was omitted from the Approved Lists of Bacterial Names and consequently has no standing in bacteriological nomenclature and was recently revived (Weiss and Schillinger, 1984).

san'francis'co. M.L. n. *sanfrancisco* San Francisco, named after the city where the sour dough from which the organism was first isolated had been propagated for more than 100 years.

Rods with rounded ends,  $0.6\text{--}0.8 \times 2\text{--}4\text{ }\mu\text{m}$ , occurring singly and in pairs.

No growth at 45°C. Does not grow reasonably in MRS broth unless freshly prepared yeast extract is added and the initial pH is lowered to 5.6. A small peptide isolated from yeast extract was found responsible for the growth-promoting effect (Berg et al., 1981).

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

DNA/DNA homology: four strains tested were found to be highly homologous among each other but showed no significant genomic relationship with *L. acidophilus*, *L. helveticus* and *L. brevis* (Sriranganathan et al., 1973). No significant homology detected with other heterofermentative lactobacilli, especially *L. confusus* and *L. fructosus* containing the same peptidoglycan type (Weiss and Schillinger, 1984) as *L. sanfrancisco*.

Isolated from sour dough.

The mol% G + C of the DNA is 36–38 ( $T_m$ ).

Type strain: NRRL B-3934.

Further comments. DNA-DNA hybridization studies have shown that other lactobacilli isolated from sour dough and labeled *L. brevis* var. *lundneri* (Spicher and Schröder, 1978) are identical with *L. sanfrancisco*.

43. *Lactobacillus vaccinofermentus* Okada, Suzuki and Kozaki 1983, 439.<sup>VP</sup> (Effective publication: Okada, Suzuki and Kozaki, 1979, 217.)

vaccino'fer'men'tus. L. adj. *vaccinus* from cows, L. n. *stercus* dung; M.L. adj. *vaccinofermentus* from cow dung.

Rods with rounded ends,  $0.9\text{--}1.0 \times 1.5\text{--}2.5\text{ }\mu\text{m}$ , occurring mostly in pairs.

No growth at 45°C.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

Growth factor requirements: thiamine, pantothenic acid, niacin, and biotin are essential; pyridoxal, *p*-aminobenzoic acid and folic acid not required.

Isolated from cow dung.

The mol% G + C of the DNA is 36 ( $T_m$ ).

Type strain: ATCC 33310.

44. *Lactobacillus viridescens* Niven and Evans 1957, 758.<sup>4L</sup> (*Lactobacillus corynoides* subsp. *corynoides* Kandler and Abo-Elnaga 1966, 573.<sup>4L</sup>)

Note. *L. viridescens* is incorrectly cited on the Approved Lists of Bacterial Names as *Lactobacillus viridescens* Kandler and Abo-Elnaga 1966, 573.

*viridescens* M.L. pres. part. *viridescens* growing green, greening.

Small, often slightly irregular rods, generally 0.7–0.9 × 2.0–5.0 µm, with rounded to tapered ends, occurring singly or in pairs.

No growth at 45°C. In contrast to the data given in the eighth edition of the *Manual*, no fermentation of ribose and gluconate could be observed in our laboratory.

Additional physiological and biochemical characteristics are presented in Tables 14.9 and 14.10.

Growth factor requirements: pantothenate, niacin, thiamine, riboflavin, and biotin are essential; folic acid and pyridoxal may be stimulatory.

DNA/DNA homology: four strains tested form a narrow homology group genetically not related to other heterofermentative lactobacilli (Vescovo et al. 1979).

Isolated from discolored cured meat products and pasteurized milk.

The mol% G + C of the DNA is 41–44 (Bd,  $T_m$ ).

Type strain: ATCC 12706.

#### Addendum I

*Lactobacillus* species included in the Approved Lists of Bacterial Names but, for reasons discussed below, not considered as belonging to the genus *Lactobacillus*.

*Lactobacillus cateniforme* (sic) (Eggerth 1935) Moore and Holdeman 1970, 15.<sup>4L</sup> (*Bacteroides cateniformis* Eggerth 1935, 286.)

*catena* for 'me. *L. n. catena* chain; *L. n. forma* form, shape; M.L. adj. *cateniforme* chainlike. Note: the correct specific epithet should read *cateniformis* because *Lactobacillus* is masculine in gender.

Small, slightly irregular rods, often in chains. Strictly anaerobic.

Good growth at 45°C. Acid is produced from amygdalin, cellobiose, esculin, fructose, glucose, glycogen, mannose, salicin, starch and sucrose; fermentation of lactose and maltose is recorded variable.

Main product from glucose fermentation is D(–)-lactic acid. No gas is produced from glucose.

Peptidoglycan of the type strain is of the Lys-Ala type (unpublished result); this peptidoglycan type is not found in other homofermentative lactobacilli.

Isolated from human feces, intestinal and pleural infections.

The mol% G + C of the DNA is 31–33 ( $T_m$ ).

Type strain: ATCC 25536.

Further comments. 16S rRNA oligonucleotide sequence studies revealed no significant phylogenetic relationship to any of the lactobacilli investigated ( $S_{AB}$  0.3; E. Stackebrandt, personal communication). The  $S_{AB}$  values for streptococci and *Clostridium innocuum* were 0.32 and 0.4, respectively. The taxonomic position of *L. cateniforme* therefore remains undetermined.

*Lactobacillus minutus* (Hauduroy, Ehringer, Urbain, Guillot and Magrou 1937) Moore and Holdeman 1972, 63.<sup>4L</sup> (*Bacteroides minutus* Hauduroy, Ehringer, Urbain, Guillot and Magrou 1937, 64.)

*minutus* L. adj. *minutus* minute, small.

Small, elliptical rods, occurring singly, in pairs and in short chains. Strictly anaerobic.

Generally no growth at 45°C. Acid is produced from glucose and variably or weakly from fructose, galactose and sucrose.

Main product from glucose fermentation is D(–)-lactic acid. No gas is produced from glucose.

Peptidoglycan of two strains studied including the type strain was of the Orn-Ser-DGlu type (unpublished result); this peptidoglycan type has not been found in other bacteria up to now.

The mol% G + C of the DNA is 45 ( $T_m$ ).

Isolated from abscesses and wounds.

Type strain: VPI 9428 (ATCC 33267).

Further comments. 16S rRNA oligonucleotide sequence studies revealed no significant phylogenetic relationship to any of the lactobacilli investigated ( $S_{AB}$  3.0; E. Stackebrandt, personal communication). The taxonomic position of *L. minutus* therefore remains undetermined.

*Lactobacillus rogosae* Holdeman and Moore 1974, 275.<sup>4L</sup>

*rogo'sae* M.L. gen. n. *rogosae* of Rogosa; named for M. Rogosa, an American bacteriologist.

Further comments. No strains which correspond to the original description are presently available. Two strains recently received from VPI, in our hands, were morphologically similar to propionibacteria; they produced mainly L(+)-lactic acid in PYG medium, but formed acetic acid and propionic acid in chopped meat media at the expense of the lactic acid naturally contained in these media. The peptidoglycan of these two strains were of the LL-DAP-Gly type, typical of propionibacteria. Moreover, the mol% G + C of 59 reported for one strain of *L. rogosae* is clearly outside the range determined in all other species of *Lactobacillus*. More investigations are needed to clarify the taxonomic position of *L. rogosae*.

*Lactobacillus xylosus* Kitahara 1938, 1449.<sup>4L</sup>

*xylo'sus* M.L. adj. *xylosus* of xylose, pertaining to xylose.

Further comments. Both nucleic acid hybridization studies (Killper-Bälz et al., 1982) and immunological investigations of fructose-diphosphate aldolase and glyceraldehyde-3-phosphate dehydrogenase (London and Chace, 1983) have shown that *L. xylosus* has not been attributed to the appropriate genus. Because of the results of DNA/DNA homology and DNA/rRNA hybridization studies, Killper-Bälz et al. (1982) stated that *L. xylosus* "should be reclassified in the same genus or even the same species as *Streptococcus lactis*." A definite statement on the taxonomic position of *L. xylosus* is required.

#### Addendum II

The following *Lactobacillus* species are not included in the Approved Lists of Bacterial Names and have not been validly described since 1980. They have, therefore, no standing in bacteriological nomenclature.

"*Lactobacillus frigidus*" Bhandari and Walker 1953, 333.

Reference strain: ATCC 11307.

"*Lactobacillus malefermentans*" Russell and Walker 1953, 162.

"*Lactobacillus lindneri*" (Henneberg 1901) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 245.

The three species are obligately heterofermentative lactobacilli and have been isolated from beer and brewery yeast. As shown by DNA/DNA homology studies, strains of the above-mentioned species are genomically not significantly related either among each other or to any other heterofermentative lactobacilli species (Vescovo et al., 1979; U. Schillinger, personal communication). They can, therefore, be regarded as additional separate species within the heterofermentative lactobacilli (group III). More comparative studies based on a greater number of strains are required for the revival of the presently invalid names.